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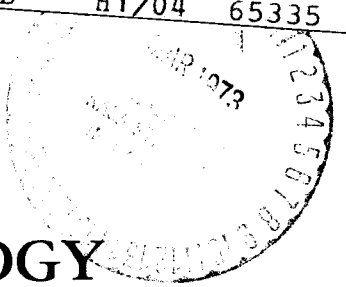

PROBLEMS OF SPACE BIOLOGY

Volume 14 - Radiobiological Aspects of the Reactivity of the Organism During Space Flights

Edited by P. P. Saksonov and B. I. Davydov

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ANNOTATION

This collection contains works on the reactivity of the organism under the influence of extremal spaceflight factors. Experimental data on the influence of radioprotectors on the ability of the organism to withstand overloads and hypoxia are examined. An analysis of the reaction of the organism to medicinal substances before and after the action of flight factors is presented. Original studies dealing with a quantitative evaluation of the recovery of reactivity under the influence of radioprotectors, radiation and dynamic flight factors are described. /4*

*Numbers in the margin indicate the pagination in the original foreign text.

FOREWORD

The problem of reactivity is of considerable scientific and practical significance not only in connection with aviation, space biology, and medicine, but generally for medical science as a whole. The experimental studies, on the one hand, allow a deeper insight into the intimate aspects of the mechanism of those functional and sometimes pathological changes which are observed as a result of the action of extremal flight factors on the organism (extreme stimuli) and, on the other hand, promote the development of rational measures intended to increase the resistance of the organism to these excessive stimuli as well as to correct functional disturbances which have already developed.

This volume contains experimental data on the study of various aspects of the reactivity of the organism.

In establishing certain standards, it is always necessary to keep in mind that under flight conditions the factors act in various combinations and may work together to increase or decrease the effect of an influence.

A strictly quantitative approach to the evaluation of the reactivity of the organism under the combined influence of penetrating radiation and non-radiation flight factors was the basis for the majority of articles in Section IV of this collection. Such data are extremely important for the development of efficient life support systems. They promote a scientifically based standardization of the limits of permissible radiation doses, the highest permissible concentrations of certain gases, harmful impurities, etc.

The experimental studies presented in this book were performed on the ground, but their value is in no way reduced by this fact. Simulation experiments together with those performed in flight will play a leading role in the future in the treatment of many questions involving space biology and medicine. /6

We express the hope that the materials presented in this book will be of interest not only to specialists in the field of space biology and medicine, but also to a wide circle of scientific workers — radiobiologists, physiologists, pharmacologists and pathophysiologists.

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I

INFLUENCE OF RADIOPROTECTIVE PHARMACOCHEMICAL PREPARATIONS ON THE REACTIVITY OF ANIMALS TO THE ACTION OF NON-RADIATION FLIGHT FACTORS

Influence of Radioprotective Preparations From the Mercaptoalkylamine Group (Cystamine, S,β-Aminoethylisothiuronium) on the Resistance of Animals to Transversely Directed Overloads

B. I. Davydov and N. A. Gaydamakin

ABSTRACT. Experiments were performed on 1,800 mice and 31 rats. The dose dependence of the influence of cystamine and AET on the resistance of mice to overloads was determined (30 minutes after administration of the preparation): $A_c = 120 \cdot \log D - 184$; $A_{AET} = 43.3 \cdot \log D - 46.6$, where A is the mortality for ten minutes of centrifuging, and D is the dose in mg/kg. The dose dependence, determined from the change in the frequency of cardiac contractions, was also observed in experiments on rats. Four hours following administration of radioprotectors, the resistance to overloads in the animals had returned to normal. The decrease in

time of the depressant effect of cystamine and AET is associated with the elimination of the pharmacological (toxic) effect. One of the possible mechanisms for the decreased resistance of the organism to overloads under the influence of cystamine is the decrease in the liver glycogen reserves.

Particularly strict requirements [4] are imposed on chemical preparations to be used as individual means of protection of the spacecraft crew members against the harmful effects of cosmic radiation. In particular, they must not reduce the resistance of the organism to overloads.

Previously [2] we studied the influence of radioprotectors from the mercaptoalkylamine group (cystamine, AET) and indolylalkylamines (5-methoxytryptamine and serotonin) in optimally radioprotective doses on the ability of the organism to withstand acceleration. It was found that these radioprotectors have a pronounced detrimental effect: resistance to overloads in animals decreases.

It was interesting in this connection to study first of all the ability to withstand overloads as a function of the dose of the preparation, i.e., to evaluate this phenomenon from the standpoint of the dose-effect and to determine the tolerable doses. In the second place, it was desirable to attempt to clarify the role of the chemical structure of the radioprotector in this phenomenon. With this goal in mind, we also studied AET for comparison with cystamine. In the third place, it was considered very important to study the pathogenetic causes for a decrease in the resistance of the organism to overloads under the influence of radioprotectors.

Experiments on mice. Thirty minutes, 1, 2 and 4 hours, and 1 - 2 days prior to centrifuging, the mice received intra-peritoneal injections of solutions of cystamine dichlorohydrate in doses of 50 - 150 mg/kg and S, β-aminoethylisothiuronium bromide of hydrobromide in doses of 50 - 150 mg/kg. /8

All of the experiments were performed in the morning (before 1200 hours), with the exception of one group of animals which received the preparation 4 hours before centrifuging. The animals were subjected to the influence of transversely directed overloads (back-chest) of 44.4 units on a centrifuge with a 4.25 m arm. The time of rotation (plateau) and the intervals between exposures amounted to five minutes.

The mortality curve for the animals subjected to centrifuging was S-shaped. If the percentage of mortality of the animals is expressed in deaths and the centrifuging time is expressed logarithmically, the experimental points will lie in a straight line.

In this case, the method of least squares can readily be used to determine the equation of regression of this relationship:

$$P = P_0 + S \cdot \log t, \qquad (1)$$

where P is the mortality (in deaths), S is the coefficient of the slope of the straight line, and t is the centrifuging time (in minutes).

Using this equation, we determine the mortality (in percent) of animals during 10-minute centrifuging. If necessary, it is also possible to calculate the average effective centrifuging time.

In Table 1, we have listed the data on the ability of the mice to withstand overloads for 30 minutes following injection of cystamine in doses of 50 - 150 mg/kg.

In column 4 of this table there is an analytical presentation of the dependence of the mortality of the animals at an overload of 44.4 units on the centrifuging time. The coefficient P_0 as follows from Equation (1), will reflect the mortality of the animals in deaths at a rotation time of 1 minute, i.e., in reality the time of the initial rise. We determined the mortality of the animals at 10-minute centrifuging intervals (A), or $A = P_0' + S'$, where P_0' and S' are the figures for the deaths converted to percentage of mortality.

The function of the slope of the deaths, curve (S), characterizes the degree of scattering and also reflects the reactivity of the animals during the centrifuging process under the stated conditions of the experiment. Hence, $A-P_0'$ will reflect the ability of the animals to withstand acceleration "on the plateau" at 10-minute centrifuging intervals.

It should be emphasized that, with fractional rotation with intervals of 5 minutes, there is an unconditional recovery of the reactivity. If we take the fact into account that this value is constant in all experiments and is not very high, we can

eliminate it from the calculations. A similar analogy may be found in radiobiological experiments when there is fractional irradiation with short intervals between the exposures and the protracted irradiation. In both cases, the effects will be similar.

As we can see from the table, the ratio of the percentage /9
of mortality of the experimental group to that for the control (A/A_0) depends on the dose of the preparation administered. Thus, at cystamine doses of 150, 100, 75 and 50 mg/kg, the A/A_0 for the males will be 2.28, 1.89, 1.16 and 0.45, respectively, while for the females it will be 2.68, 2.38, 1.89 and 0.73, respectively.

From these data it is readily apparent that the ability to withstand acceleration under the influence of cystamine decreases to a greater extent in the females than in the males. It is interesting that this phenomenon has nothing to do with the fact that the control females were less resistant to overloads than the males. On the contrary, as indicated by the table, the mortality of the male mice in the control groups was higher than in the same groups for the females: 32.0 $\pm$ 2.5% (36.9 - 27.1, $p = 0.05$, $n = 340$ animals) and 23.9 $\pm$ 3.5% (30.7 - 17.1, /10
$p = 0.05$; $n = 150$ animals), respectively.

Hence cystamine, so to speak, smooths out the sexual differences as far as ability to withstand overloads is concerned, and the relatively lower resistance of the females is due to their higher resistance in the control groups.

Figure 1 shows the relationship between the cystamine dose and the ability of the mice to withstand overloads. Using the method of least squares, we established a correlation between

TABLE 1

INFLUENCE OF CYSTAMINE ON ABILITY OF MICE TO WITHSTAND OVERLOADS

Group	Dose, mg/kg	Number of animals	$P=P_0+S\cdot lg\ t$*	Mortality (in %) with t=10	A/A_c	S/S_0
			MALES			
Control	0	180	P=3.46+1.08 lg t	32.2±3.4	—	—
Cystamine	150	50	P=3.51+2.10 lg t	73.0±6.4	2.28	1.96
Control	0	80	P=2.75+1.71 lg t	29.5±5.1	—	—
Cystamine	100	80	P=3.43+1.73 lg t	56.5±5.5	1.89	1.01
Control	0	20	P=1.75+2.70 lg t	29.0±10.2	—	—
Cystamine	75	20	P=1.87+2.70 lg t	33.6±10.5	1.16	1.00
Control	0	60	P=3.24+1.40 lg t	36.0±6.2	—	—
Cystamine	50	60	P=2.77+1.25 lg t	16.3±4.7	0.45	0.88
			FEMALES			
Control	0	50	P=2.67+1.68 lg t	25.8±6.0	—	—
Cystamine	125	50	P=3.82+1.68 lg t	69.2±6.4	2.68	1.0
Control	0	40	P=3.00+1.26 lg t	23.0±6.7	—	—
Cystamine	75	40	P=2.92+1.90 lg t	42.8±7.7	1.89	1.51
Control	0	40	P=2.13+2.10 lg t	22.0±6.5	—	—
Cystamine	50	40	P=1.90+2.10 lg t	16.0±5.7	0.73	1.00
Control	0	20	P=1.89+2.30 lg t	21.2±9.3	—	—
Cystamine	100	20	P=2.71+2.30 lg t	50.4±11.5	2.38	1.00

Note. A - trials, expressed in % at t = 10 minutes for experimental animals;
A_0 - ditto, controls.

*lg appears in place of log.

the mortality of the animals and the logarithm of the dose of cystamine.

$$A = 120 \cdot \log D - 184 \qquad (2)$$

where A is the mortality (in percent) of the animals with

10-minute rotation; D is the cystamine dose (in mg/kg).

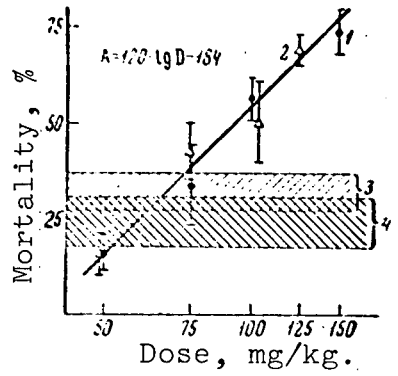

Figure 1. Relationship between dose (D) of cystamine and mortality (A) of mice and overloads (44.4 units, 10 minutes). 1 and 3 - males; 2 and 4 - females.

The differences in the equations of regression between the males and females were insignificant. According to the equation of regression, we can determine the threshold cystamine doses:

For males with A = 32 (36.9 - 27.1) the percentage of the dose is 63 (69 - 57) mg/kg and for females with A = 23.9 (30.7 - 17.1) % - 54 (62 - 47) mg/kg).

Consequently, the threshold doses of cystamine on the basis of the criterion of tolerance of overloads by the mice will amount to 54 - 63 mg/kg.

Specific information regarding the resistance of the organism to overloads may also be provided by the mortality rate of the animals during the rotation. This index characterizes to a certain degree the adaptation mechanisms of the organism with time at a constant force of the influence factor, in this case, with a constant overload. This index, in our opinion, reflects primarily the mobility of the energy reserves of the organism, the lability of the tissues toward hypoxia, which develops during overloads.

A changing reaction of the animals during the centrifuging period is reflected by the function of the slope of the mortality

curve, i.e., the value S. Thus, in the case of males at a dose of 150 mg/kg, the value S/S_0 is higher than for the control animals. It approaches unity at 100 - 75 mg/kg, and becomes slightly less at 50 mg/kg (Table 2). In the females, this coefficient was equal to unity at all cystamine doses, with the exception of the 75 mg/kg dose (1.51). /11

The coefficient P_0 is also of interest in conjunction with understanding the mechanism of withstanding overloads. At $t = 30$ sec (initial rise) the mortality determined by formula (1) will characterize the resistance of the animals during the rise to the "plateau". However, for convenience it is better to let $t = 1$ minute, so that $P = P_0$.

As we can see from the data presented, the coefficient P_0, although it is insignificant, is slightly higher in the experimental groups with cystamine doses of 150 - 100 mg/kg. This indicates a slight decrease in the adaptive regulatory mechanisms of the cardiovascular system during increased overloads.

In subsequent experiments, we attempted to measure the recovery period of normal reactivity of the mice with respect to overloads at cystamine doses of 100 and 150 mg/kg.

Table 3 shows the results of a study dealing with the change in ability of mice to withstand acceleration for a period of four hours to 2 days after injection of cystamine at a dose of 150 mg/kg, while Table 4 shows the same thing at a cystamine dose of 100 mg/kg. The average mortality value for the control animals according to these data amounts to 32.4 ± 2.2 (28.1 - 36.7; $p = 0.05$; $n = 340$ animals). This value differed slightly from the one which was found from the data in Table 1.

TABLE 2

RELATIONSHIP OF THE P_0 COEFFICIENT TO THE PREPARATION DOSE

Sex	Dose, mg/kg	Experiment (P_0')	Control (P_0)	P_0'/P_0	Sex	Dose, mg/kg	Experiment (P_0')	Control (P_0)	P_0'/P_0
Males	150	3.51	3.46	>1	Females	125	3.82	2.67	>1
	100	3.43	2.75	>1		100	2.71	1.89	>1
	75	1.87	1.75	>1		75	2.92	3.00	<1
	50	2.77	3.24	<1		50	1.9	2.13	<1

TABLE 3

INFLUENCE OF CYSTAMINE (150 mg/kg) ON THE ABILITY OF MICE (MALES) TO WITHSTAND OVERLOADS

Group	Interval between injection of cystamine and overload	Number of animals	$P=P_0+S\cdot lg\ t$*	Mortality (in %) with t=10	A/A_0	S/S_0
Control	—	90	$P=2.70+1.44\ lg\ t$	19.6±4.2	—	—
Experiment	4 hours	67	$P=3.81+1.21\ lg\ t$	50.6±6.1	2.56	0.84
Control	—	180	$P=3.46+1.08\ lg\ t$	32.2±3.4	—	—
Experiment	1 day	38	$P=3.16+1.34\ lg\ t$	31.0±7.5	0.96	1.23
Control	—	20	$P=1.60+2.50\ lg\ t$	18.4±7.2	—	—
Experiment	2 days	20	$P=2.34+2.10\ lg\ t$	28.8±10.2	1.57	0.84

*lg appears in place of log.

TABLE 4

INFLUENCE OF CYSTAMINE (100 mg/kg) ON THE ABILITY OF MICE (MALES) TO WITHSTAND OVERLOADS, 1-4 HOURS AFTER INJECTION OF THE PREPARATION

Group	Interval between injection of cystamine and overload	Number of animals	$P=P_0+S \cdot \lg t$*	Mortality (in %) with t=10	A/A_0	S/S_0
Control	—	60	$P=2.60+1.56 \lg t$	20.0 ± 4.3	—	—
Experiment	1	20	$P=1.73+2.97 \lg t$	38.2 ± 11.0	1.90	1.9
	2	20	$P=1.99+1.94 \lg t$	13.6 ± 5.6	0.68	1.24
	4	20	$P=2.22+1.75 \lg t$	15.2 ± 5.9	0.76	1.12

* lg appears in place of log.

As we can see from Figure 2 (Curve 1), when cystamine is administered at a dose of 150 mg/kg, the resistance of the mice to overloads reaches the control level in 12 hours (interpolated value), while at a dose of 100 mg/kg it reaches this level only 1 hour after injection of the radioprotector. In addition, after 2 to 4 hours there is even an increase in the resistance to overloads.

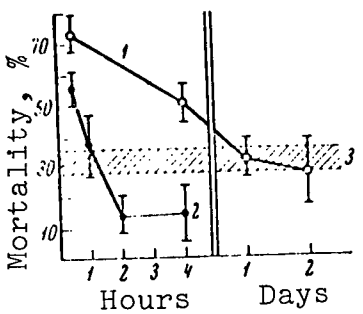

Figure 2. Change in resistance of mice (male) to overloads (44.4 units, 10 minutes) as a function of the time interval between the injection of the cystamine in doses of 150 mg/kg (1) and 100 mg/kg (2) and centrifuging.
3-% of mortality of control animals with reliable intervals (p=0.05)

As we said earlier, the value A reflects the total mortality during the rise and on the overload "plateau" for 10 minutes of rotation. Basically, coefficient S obviously reflects the mortality /12 of the animals on the centrifuging plateau.

At a dose of 150 mg/kg, S reliably increases the level of the controls only in the group with an interval of 30 minutes between the injection of the radioprotector and the rotation. At a dose of 100 mg/kg, a considerable increase of S above the control level is seen only 1 hour following injection of cystamine. Although at this dose the value of A differs insignificantly from the control value, the value S/S_0, as we can see from Table 3, is rather high. This indicates that the ability to withstand overloads during centrifuging is higher on the plateau and lower during the initial rise (P_0) than in the control mice.

The data shown in Figure 2 may be approximated by a straight /13 line if the mortality of the animals is expressed in percent on a

linear scale, and the time following injection of the preparation is expressed on the logarithmic scale:

$$A = a - b \cdot \log t,$$

where t is the time following injection of the preparation in minutes and a and b are coefficients.

For a cystamine dose of 150 mg/kg, the equation of regression will have the form A = 114 - 27 log t, while for a dose of 100 mg/kg it will be A = 119 - 45.5 log t. On the basis of these equations, we can determine the time interval between the injection of the cystamine and the overload at which mortality will be within the limits of the control level ($A-A_0$). For a dose of 100 mg/kg, t is 67 - 100 minutes, and for a dose of 150 mg/kg it will be 750 - 1500 minutes (12.5 - 27 hours).

The threshold doses for various time intervals following injection of radioprotectors amounted to 63 mg/kg for 20 - 30 minutes, 100 mg/kg for 67 - 100 minutes, and 150 mg/kg for 12.5 - 27 hours (Figure 3).

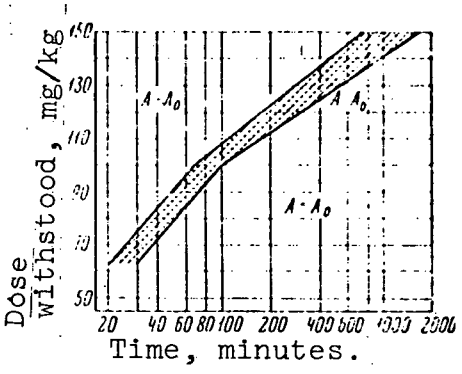

Figure 3. Threshold doses of cystamine for various time intervals following its injection.

Examining the influence of cystamine on the ability to withstand overloads after various intervals of time following injection of radioprotectors, we assumed that recovery of normal reactivity in this case is apparently related to the elimination of the pharmacological effect of cystamine. In addition, it is necessary to take into account the influence of the radioprotector on the recovery of the organism reactivity following a subthreshold overload.

The method used in this experiment amounts to the following: 20 - 30 minutes prior to centrifuging of the animals (female mice), they received an intraperitoneal injection of cystamine (150 mg/kg). Thirty minutes later, they were subjected to /14 centrifuging (g_1) with an overload of 30 units for 5 minutes. At the overload level, it was shown in preliminary experiments that the death of the animals amounts to 1 - 2%. After 30 minutes, 4 hours and 1 day following the first centrifuging, the animals were subjected to a second overload (44.4 units) (g_2).

Table 5 summarizes the results of this experiment. As we can see, cystamine reduces the recovery rate of organism reactivity following the action of a subthreshold overload. It it interesting to note that the slowing down of the recovery of mice reactivity to the second overload under the influence of cystamine is apparently caused by the action of this preparation on the compensatory possibilities of the cardiovascular system. Death following centrifuging for one minute (interpolated value) is higher in groups where cystamine was admistered beforehand: thus, for a 30-minute interval, this value is 31.2% for animals which received cystamine, and 0.3% under the influence of a single overload (without cystamine).

As we know, cystamine and cysteamine have a pronounced glycolytic effect [1, 6, 9]. They reduce the glycogen in the liver particularly sharply, while its content in the heart muscle remains practically constant [1]. Figure 4 shows data which illustrate the change in the glycogen content in the liver following an overload at a 30 unit level, and following the combined action of cystamine and overload.

Between curve 2 (resistance of the animals to repeated rotation) and curve 5 (content of glycogen in the liver) there is

TABLE 5

INFLUENCE OF CYSTAMINE (C) (150 mg/kg) ON THE RECOVERY OF REACTIVITY BY MICE FOLLOWING EXPOSURE TO OVERLOADS: g_1 = 30 units, 5 minutes, g_2 = 44.4 units.

Group *	Interval, g_1 - g_2	$P=P_0+S\cdot lg\ t$**	Mortality (in %) with t = 10 minutes
C + g_1 + g_2	30 minutes	P=4.51+0.94 lg t	66.2±11.3
g_1 + g_2	30 minutes	P=2.28+2.50 lg t	41.2±11.2
C + g_1 + g_2	4 hours	P=3.97+0.90 lg t	45.0±11.4
g_1 + g_2	4 hours	P=1.50+2.60 lg t	20.1± 9.1
C + g_1 + g_2	1 day	P=2.35+2.45 lg t	41.0±11.2
g_1 + g_2	1 day	P=3.0+0.90 lg t	13.6± 7.6
g_2	—	P=4.02+0.42 lg t	28.8±10.3

*20 Animals in each group.

**lg appears in place of log.

an inverse relationship. In combination with overloads, cystamine leads to a more pronounced decrease in the glycogen reserves in the liver. It is interesting that succino-dehydrogenase, which plays an important role in the citric acid cycle, initially decreases insignificantly during overloads, but after 4 to 6 and 24 hours the activity of this enzyme increases. Under the influence of cystamine, there is likewise an initial decrease in this enzyme, but later there is an increase and subsequent decrease (after 24 hours). Hence, while conjugate relationships are observed between the activity of this enzyme and the glycogen content in the liver during overloads, the opposite type of change is observed under the influence of cystamine. If we perform a formal comparison of the enzymatic changes with the resistance of the animals to overloads, we will conclude that the conjugate changes (in terms of direction) of the activity of succino-dehydrogenase and the glycogen content in the liver

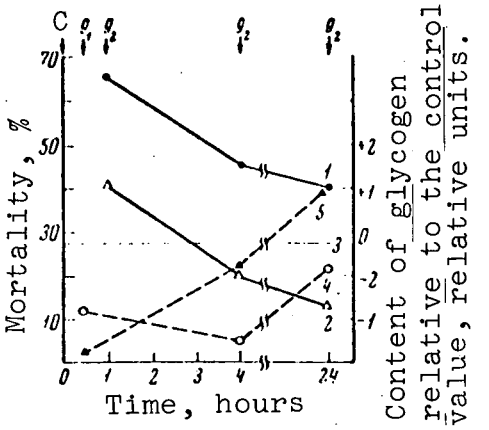

Figure 4. Influence of cystamine on the recovery of reactivity in mice following centrifuging. 1 - cystamine (150 mg/kg) + g_1 + g_2; 2 - g_1 + g_2; 3 - g_2; 4 - glycogen in the liver following cystamine and overload; 5 - glycogen following a single centrifuging (30 units, 5 minutes).

are adaptative, ensuring higher resistance to repeated overloads.

If we compare Curves 1 and 2 in Figure 4, we can see that the changes in the resistance of the animals to a repeated overload with time are quite similar, although when a second injection of cystamine is given (Curve 1, Figure 4) the recovery of reactivity proceeds more slowly. Here, obviously, we can assume that the overload slows down the elimination of the cystamine (or its metabolites) from the organism.

We have already said that the recovery of reactivity of animals to overloads under the influence of cystamine takes place after 4 hours. /16

In addition, the converse activity of the preparation decreases more rapidly than normalization of the reactivity of the organism to overloads takes place. The impression is created that the decrease in the resistance to overloads is caused not only by the cystamine itself, but by its metabolites as well. We know that cystamine, when it enters the blood, is rapidly converted into cysteamine, which ceases to be evident in the blood after a short time [5, 7, 8]. A. S. Mozzhukhin [3], analyzing the data of A. V. Titov [5], concludes that the cysteamine is either destroyed or combines with proteins.

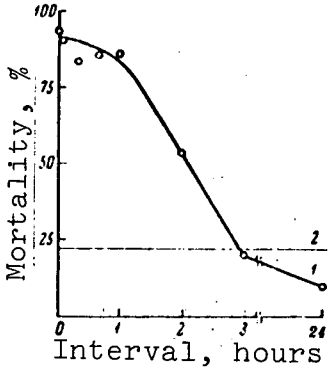

Figure 5. Mortality of mice with double injection of cystamine at various intervals.

1 - 125 + 225 mg/kg;
2 - 225 mg/kg, single dose.

It was interesting to observe the behavior of cystamine and the organism using the method of elimination of the pharmacological effect. This term, which we shall use, may not exactly reflect the internal essence of the behavior of cystamine but, in our opinion, it is adequate as far as determining the external aspect of this process is concerned. Using the general idea of quantitative estimation of the recovery from radiation damage, we attempted to determine the elimination of the radioprotector in the organism.

In experiments performed on 250 mice, the animals received an intraperitoneal injection of cystamine (125 mg/kg). Repeated doses of cystamine (225 mg/kg) were given to each group of animals at intervals of 5, 20, 40 minutes and 1, 2, 3, 8 and 24 hours later, respectively. As we can see from Figure 5, death decreases following the second injection of cystamine with an increased time interval and after 3 hours we can see that the effect of the first dose has been completely eliminated: by a double injection (125 + 225 mg/kg), with an interval of 3 hours, the mortality was 20%. With a single injection of cystamine at a dose of 225 mg/kg, it was 22%.(1)

(1) The method is described in more detail in the article by B. I. Davydov, "Elimination of Cystamine in the Organism and the Prolongation of its Radioprotective Effect". (This collection, Page 222).

Hence, we can say that the state of activity of the mice with respect to overloads is correlated to some extent with the elimination of the pharmacological effect.

The reasons for the differences between the decrease in the radioprotective effectiveness of cystamine and its excretion (on the basis of the determination of SH-groups) — on the one hand by the elimination of the pharmacological effect and the recovery of the ability to withstand overloads by the organism, on the other — are still not clear.

Apparently an important role in the damping effect (on the basis of the overload tolerance criterion) of radioprotectors must be attributed to its chemical structure. Earlier [2] we reported that at the optimally protective dose of AET (150 mg/kg), the resistance of animals to overloads decreases. However, this decrease was less pronounced (Table 6) than for cystamine 30 minutes to 1 day following injection of a radioprotector. /17

Figure 6 shows the ability to withstand overloads as a function of the dose of s,β-aminoethylisothiuronium. As in the case with cystamine, it may be expressed by a linear-logarithmic relationship:

$$A = 43.3 \cdot \log D - 46.6$$

where A is the mortality of the animals (in percent) and D is the dose of radioprotector.

The line of regression of this equation intersects the zone of the controls (41 - 27.4%, $p = 0.05$) at doses of 105 and 50 mg/kg (average value - 74 mg/kg). As we can see, the range of tolerable doses is much wider than for cystamine. This has to do with the fact that the coefficient of regression "dose-effect" is less for AET than for cystamine.

TABLE 6

TOLERANCE OF OVERLOADS BY MICE AS A FUNCTION OF DOSE AND TIME OF ADMINISTRATION OF AET

Group	Number of animals	Dose, mg/kg	Time interval between AET and overload	$P=P_0+S\cdot\log t$	Mortality (in %) at t = 10	A/A_0
Control	200	—	—	P=3.4+1.20 log t	34.4±3.4	—
Experiment	30	150	30 min.	P=3.86+0.99 log t	44.0± 9.0	1.28
	20	100	30 min.	P=1.57+3.35 log t	47.0±11.5	1.36
	20	50	30 min.	P=2.25+2.10 log t	25.8±10.0	0.75
	20	150	1 day	P=3.30+1.02 log t	24.2±10.4	0.7
Control*	90	—	—	P=2.70+1.44 log t	19.6±4.2	—
Experiment*	39	150	4 hours	P=2.94+1.36 log t	24.2±6.5	0.7

* Centrifuging performed during the second half of the day (14 - 16 hours).

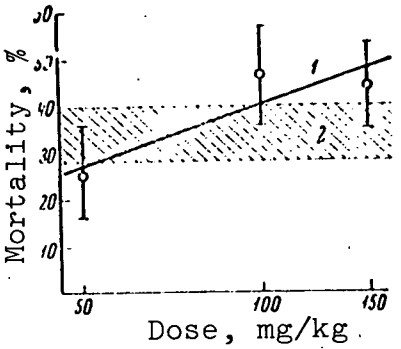

Figure 6. Mortality of male mice during centrifuging with various doses of AET (1); 2 - control.

S,β-aminoethylisothiuronium, although it belongs (like cystamine) to the mercaptoalkylamines, is a derivative of thiourea.

It is also clear that the difference between AET and cystamine, on the basis of the ability to withstand overloads, is governed by their different pharmacological effect as well as by metabolism.

In our opinion, it is completely possible to assume that the stronger effect of cystamine relative to AET on the resistance of animals to withstand overloads has to do with the presence of free SH-groups. A. S. Mozzhukhin and F. Yu. Rachinskiy [3] emphasize: ".... that the preparations from the cysteamine-cystamine group frequently produce pharmacological effects that are opposite /18 to the effect of preparations of the S,β-aminoethylisothiuronium-mercaptoethylguanidine group". (Page 207).

Experiments on Rats. Rats were subjected to transversely directed overloads (chest-back) on the same centrifuge as the mice. The magnitude of the overload was 30 units, with a rise and fall time of 30 seconds (1 unit per second); the duration of the rotation was 5 minutes. During centrifuging, the electrocardiogram was recorded from leads I, II, and III. The rhythm of cardiac activity was subjected to statistical analysis on a computer.(2) We determined the average value (M) by groups,

(2) We would like to use this opportunity to express our gratitude to Candidate in Technical Sciences V. K. Filosov for the possibility of performing our statistical analysis of the material on a computer.

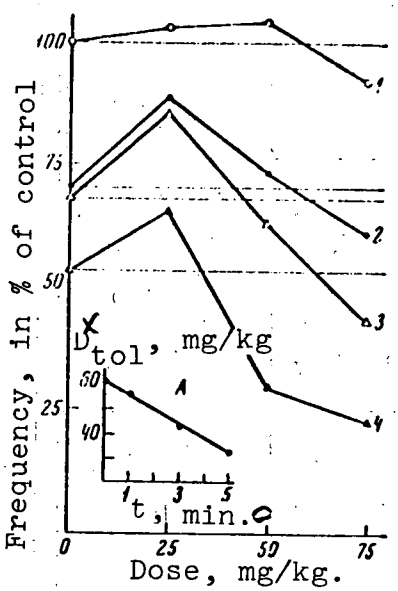

Figure 7. Change in frequency of cardiac contractions during the first (2), third (3) and fifth (4) minutes of centrifuging (30 units, 5 minutes) as a function of the dose of cystamine. 1 - cystamine without overload; A - relationship of the tolerated dose to the centrifuging time.

the mean square error (m), the coefficient of variation (v), asymmetry (A_s) and the excess (E) of distribution of frequencies in each group prior to centrifuging, during the action of acceleration, and during the period of aftereffects.

Fifteen minutes prior to centrifuging, one group of rats received an intraperitoneal injection of cystamine dichlorohydrate in doses of 25, 50 and 75 mg/kg (calculated for the base). Thirteen rats were subjected to control centrifuging, while 18 animals received the preparation. The experiments were performed only on the males. In the control group, following 5 minutes of centrifuging at an overload of 30 units, 61.5% of the animals remained alive following injection of cystamine in doses of 25, 50 and 75 mg — 84.0, 50.0 and 25%, respectively. In the rats as well as in the mice, there was a dose dependence. However, at a dose of 25 mg/kg the survival rate was even slightly higher than in the control rats. We attempted to analyze in greater detail the changes in the rhythm of cardiac activity during the period of centrifuging under the influence of cystamine. Table 7 shows some of the statistical characteristics of cardiac rhythm during the period of centrifuging and 1 to 3 minutes following the action of the overloads. Under the influence of cystamine (15 minutes after injection), the frequency of cardiac

TABLE 7

INFLUENCE OF CYSTAMINE ON THE FREQUENCY OF CARDIAC CONTRACTIONS UNDER THE INFLUENCE OF OVERLOADS (30 Units)

Dose of cystamine, mg/kg	Number of animals	Statistical indices	Frequency of Cardiac Contractions, Beats/Min								Survival, %
			Original	Centrifuging time, min					After centrifuging, min.		
				1	2	3	4	5	1	3	
Control	13	M	554.6	385.2	386.9	380.7	319.1	298.3	324.9	309.1	
		$+\sigma$	42.1	150.4	156.2	164.7	160.9	180.7	194.5	207.8	
		$\pm m$	11.6	41.7	43.3	45.6	46.4	52.1	56.1	60.0	61.5
		v, %	7.6	39.0	40.4	43.3	50.4	60.6	69.8	67.2	
		A_s	-0.39	-0.63	-0.48	-0.67	-0.35	-0.13	-0.54	-0.42	
		E_s	-1.60	-1.08	-1.52	-1.53	-1.73	-2.00	-1.80	-1.68	
75	8	M	513.7	338.7	287.4	237.4	178.8	127.1	167.1	151.4	
		$+\sigma$	40.6	121.3	125.4	121.8	99.0	85.7	154.5	158.5	
		$\pm m$	14.3	42.9	44.3	43.0	37.4	32.4	58.4	59.9	
		v, %	7.9	35.8	43.6	51.3	55.4	67.5	92.4	104.7	25.0
		A_s	-0.19	-0.57	-0.16	+0.70	+0.51	+0.56	+0.74	+0.78	
		E_s	-1.61	-1.03	-1.72	-1.47	+0.59	+0.67	-1.53	-1.45	
25	6	M	571.6	486.5	478.3	473.3	401.3	362.6	401.6	349.9	
		$+\sigma$	41.1	56.0	68.8	78.5	153.6	168.9	90.8	28.7	
		$\pm m$	16.8	22.9	28.0	32.0	62.7	68.9	37.0	11.7	
		v, %	7.2	11.5	14.4	16.5	38.3	46.6	22.6	8.2	84.0
		A_s	+0.58	+0.39	-0.53	-0.85	-0.43	-0.47	-0.56	-1.08	
		E_s	-1.53	-1.31	-1.19	-0.87	-1.86	-1.89	-0.85	-0.50	

contractions increases slightly at doses of 25 to 50 and decreases at a dose of 75 mg/kg.

Figure 7 shows a family of curves reflecting the changes in /23 heart rhythm during the first, third and fifth minutes of centrifuging as a function of the dose of the preparation administered. Here we can also see the dose dependence of the changes in the frequency of cardiac contractions upon the rotation time.

An attempt could be made to determine the tolerable dose of cystamine for various centrifuging times by determining the frequency of the cardiac contractions equal to the original value. In Figure 7, this point corresponds to the intersection of the curves with the lines of the original value. The dependence of the tolerable dose (D_{tol}, mg/kg) on the centrifuging time (t, min) may also be expressed by the equation $D_{tol} = 60.0 - 5.0 \cdot t$ ($t \leqslant 5$) (Figure 7, A).

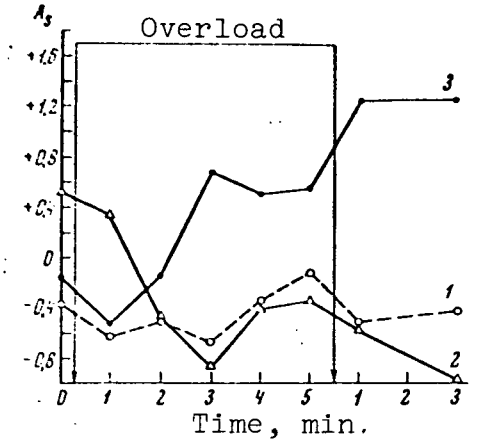

Figure 8. Change in coefficient of asymmetry (A_S) of frequency of cardiac contractions during centrifuging of control rats (1) and after preliminary administration of cystamine 25 mg/kg (2) and 75 mg/kg (3).

Table 8 shows the ranges of tolerable doses with consideration of the coefficient of variation for various periods of centrifuging. At a dose of 25 mg/kg, the coefficient of asymmetry during the period of centrifuging differs slightly from A_s of the control animals (Figure 8). At the same time, however, at a dose of 75 mg/kg, beginning with the second minute of rotation, the coefficient of asymmetry increases until the centrifuge stops.

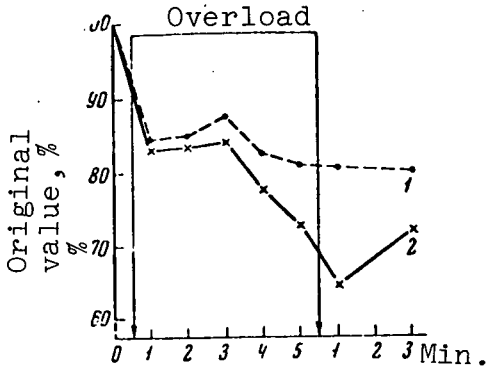

Figure 9. Dynamics of frequency of cardiac contractions under the influence of centrifuging in surviving control rats (1) and after preliminary administration of cystamine (25 mg/kg) (2).

All of what we have said above applies to the analysis of the cardiac rhythm in the rats which survived and those which died, combined into a single general group. In Figure 9, we have plotted two curves which reflect the dynamics of the frequency of cardiac contractions during the period of centrifuging and after its influence in the surviving control and experimental rats (cystamine dose of 25 mg/kg). As we can see from these curves, the decrease in frequency of cardiac contractions during the period of centrifuging following injection of cystamine at a dose of 25 mg/kg is higher than in the control rats. Thus, during the fifth minute of exposure, the frequency of the cardiac contractions in the control rats was 82% of the original level, while in the experimental ones it was 72%. Following the overload, the experimental animals showed a decrease in the cardiac activity to 38%, which was not observed in the control rats. This indicates that, even at a dose of 25 mg/kg, the reactivity

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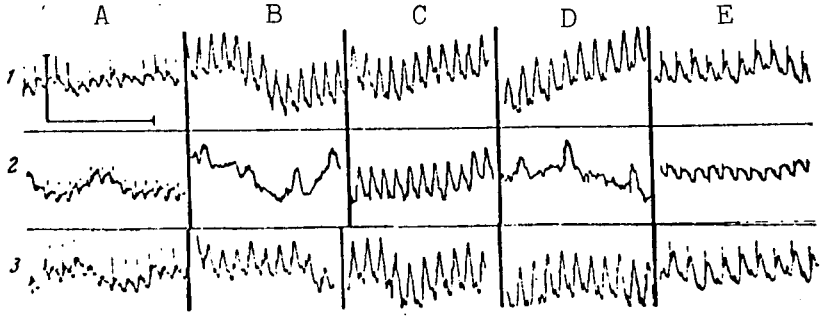

Figure 10. Changes in EKG (second lead) in rats during 30-unit overload in controls (1) and under influence of cystamine: 50 mg/kg (2) and 25 mg/kg (3). A - before centrifuging. B, C, D — 1st, 3rd and 5th minutes of action; E — 3rd minute following action.

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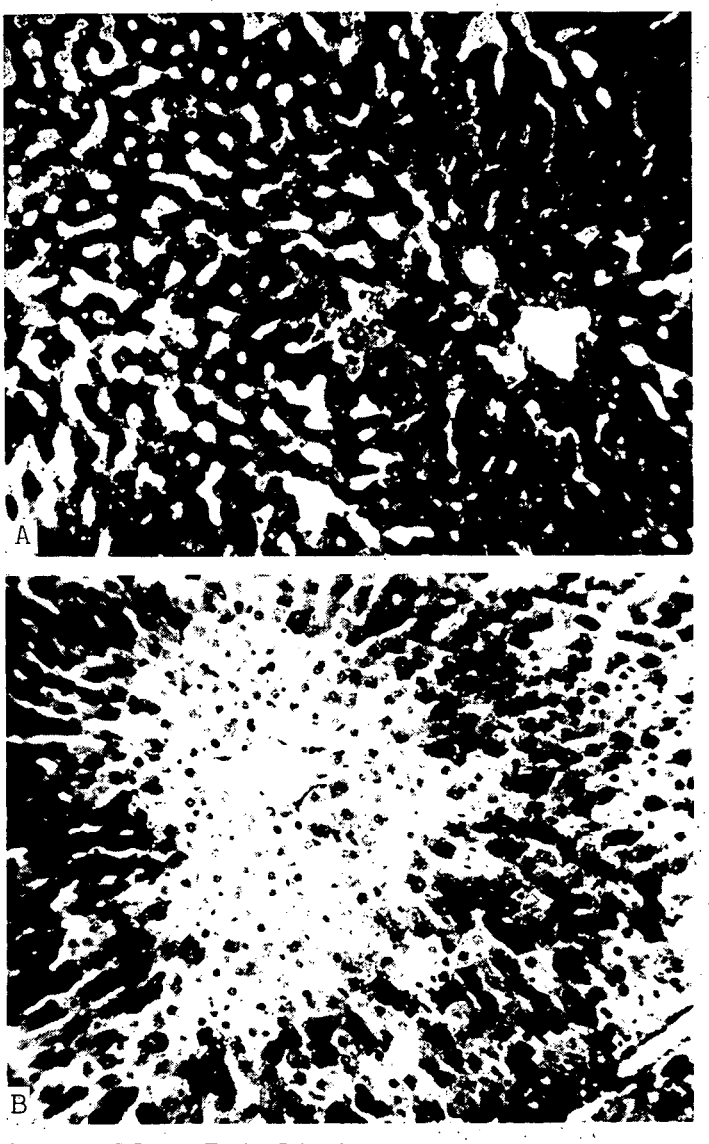
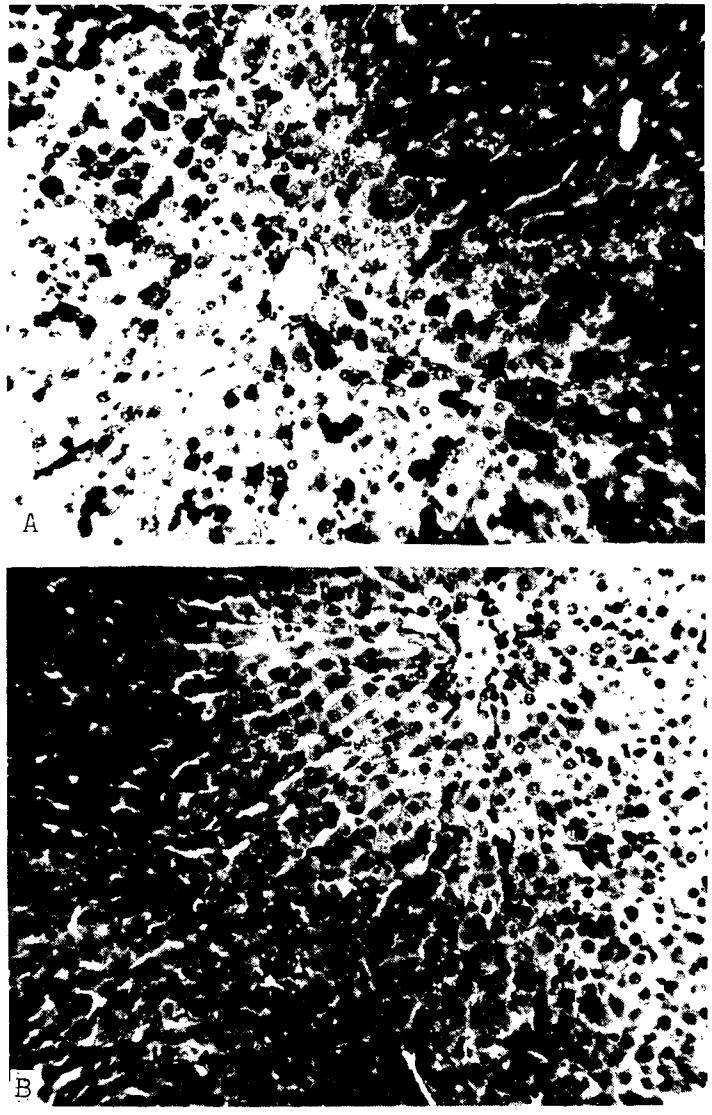


Figure 11. Rat liver. A — usual content of glycogen. B — glycogen content reduced 30 minutes after influence of overload (30 units, 5 minutes). Shabadash, Gomal', IV, ob. 16.

of the rats with respect to prolonged overloads is slightly less than in the control animals, although at this dose level the cystamine has an insignificant influence on the qualitative characteristics of the electrocardiogram of the rat under the influence of overloads in comparison to the control (Figure 10).

All of the rats which survived the centrifuging were killed for pathomorphological and histochemical examination. In the histochemical examination of the liver, we found a sharp increase in the content of fat droplets in the cytoplasm of the liver cells in the animals of all groups regardless of the conditions to which they had been exposed.



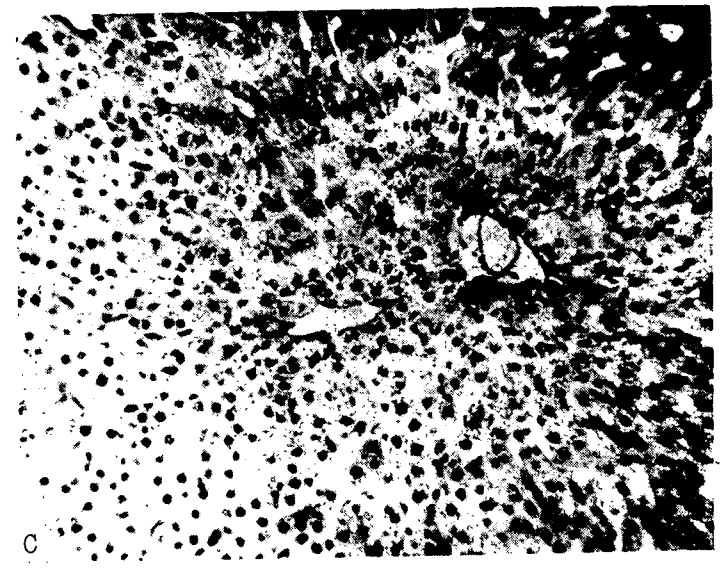


Figure 12. Content of glycogen in rat liver 30 minutes after exposure to overloads with preliminary administration of cystamine. A — dose equals 25 mg/kg, considerable reduction of glycogen; B — dose equals 50 mg/kg, almost complete disappearance of glycogen; C — dose equals 75 mg/kg, glycogen completely absent. Shabadash, Gomal' IV, OB.16.

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TABLE 8

TOLERATED DOSES OF CYSTAMINE (BASED ON THE CHANGE IN FREQUENCY OF CARDIAC CONTRACTIONS) FOR RATS AT VARIOUS CENTRIFUGING TIMES WITH AN OVERLOAD OF 30 UNITS

Time of Centrifuging, Minutes	Tolerated Dose, mg/kg	v (%)*	Range of Tolerated Doses, mg/kg
0 (without overload)	60	6	56.4 — 63.6
1	55	30	38.6 — 71.5
3	45	33	30.2 — 59.8
5	35	39	21.4 — 48.6

* The interpolated value of the coefficient of variation between 2 adjacent doses.

The content of glycogen in the liver of the surviving animals 30 minutes after centrifuging decreased significantly (Figures 11, 12). Under the combined influence of cystamine and overloads, the decrease in glycogen was more noticeable, especially when the dose of cystamine was increased from 25 to 50 and 75 mg/kg.

On the basis of the experimental data obtained, we can formulate the following four principal conclusions:

(1) there is a dose dependence of the influence of cystamine and AET on the resistance of the organism to overloads;

(2) the beneficial effect of these radioprotectors decreases with time; this apparently has something to do with the elimination of the pharmacological effect;

(3) AET, in comparison to cystamine, has a gentler effect, which apparently has something to do with the absence of free SH-groups; /25

(4) one of the possible mechanisms for the reduction of resistance of the organism to overloads under the influence of cystamine is the decrease of the glycogen supply in the liver as one of the sources of energy formation.

The experimental data presented in this paper in connection with determination of the permissible doses of cystamine and AET according to the overload tolerance criterion may be applied to man. In general, the selection of the criteria for extrapolation of experimental data to man is very complicated and requires a certain number of assumptions. In addition, it has limited conditions for its application.

An analysis of the data obtained from this standpoint will be the subject of our future papers.

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Influence of Monosodium Salt of β-Aminoethylthiophosphoric Acid on the Resistance of Animals to Transversely Directed Overloads

/25

B. I. Davydov and V. A. Kozlov

ABSTRACT. The experiments were performed on 760 mice. After 30 minutes, 4 hours, 1, 2 and 5 days following intraperitoneal administration of tsistafos, the animals were subjected to overloads (44 units). The resistance of the animals to the overloads 30 minutes following administration of tsistafos is a function of the dose described by the equation of regression

$$\log A \text{ (males)} = 1.005 + 0.00221 \cdot D$$

and

$$\log A \text{ (females)} = 0.785 + 0.00221 \cdot D$$

where A is the death of animals after ten minutes of centrifuging, D is the preparation dose (in mg/kg). Four hours and 1 - 5 days following administration of tsistafos at a dose of 300 mg/kg, the death of animals following centrifuging did not differ significantly from the death of the control animals.

It was shown in the previous article by B. I. Davydov and N. A. Gaydamakin that cystamine and AET have slightly different action as far as resistance of animals to overloads is concerned.

The suggestion was made that this phenomenon is caused by the chemical structure of the radioprotector, in particular the presence of mercapto groups in them.

It was interesting to determine the influence of some other radioprotector from the group of aminothiols, which have less toxicity and consequently perhaps a lesser influence on the decrease of resistance of the animals to overloads. /26

From this standpoint, it seemed to us that it would be interesting to study a radioprotector such as the S-derivative of mercamine — the monosodium salt of β-aminoethylthiophosphoric acid ("tsistafos") [3]. According to the data of G. M. Ayrapetyan [1], tsistafos increased the survival rate of irradiated mice up to 86 - 90%. This compound was found to be 2.5 - 3.5 times less toxic than cystamine; LD_{50} with intraperitoneal injections for mice was 930 mg/kg. /27

Experiments to study the influence of this radioprotector on the resistance to overloads were performed on 760 mice of both sexes. Tsistafos was administered intraperitoneally in doses of 100 - 450 mg/kg (as the anhydrous salt) 30 minutes, 4 hours, 1, 2 and 5 days prior to centrifuging. The parameters of rotation and the method of processing the experimental material have been described previously [2].

In Tables 1 and 2 we show the experimental data on the ability of mice to withstand overloads (Table 1 and 2). An analysis of the experimental material revealed that as the preparation dose increased, the ability to withstand overloads deteriorated. This relationship may be expressed by the equation of regression:

$$\log A = \log A_0 + b \cdot D,$$

TABLE 1

INFLUENCE OF TSISTAFOS ON TOLERANCE OF OVERLOADS BY MICE (MALES)

Group	Number of animals	Time interval between injection of preparation and rotation	Dose, mg/kg	$P=P_0+S\cdot\log t$	Mortality (in %) at t = 10	A/A	S/S_0
Control	20	—	—	P=1.26+3.50 log t	40.4±11.2	—	—
	20	30 min.	100	P=2.60+1.72 log t	24.8±10.2	0.61	0.49
Experiment	20	30 min.	260	P=3.70+1.15 log t	40.0±11.2	0.99	0.30
	20	30 min.	400	P=3.76+2.58 log t	91.0± 7.0	2.25	0.73
Control	20	—	—	P=2.86+1.55 log t	27.6±10.3	—	—
Experiment	30	30 min.	200	P=2.82+1.34 log t	20.0± 7.3	0.72	0.86
Control	50	—	—	P=2.72+1.85 log t	33.2± 6.5	—	—
Experiment	50	30 min.	300	P=3.35+1.34 log t	38.0± 6.7	1.14	0.73
Control	40	—	—	P=3.25+1.20 log t	29.0± 7.3	—	—
Experiment	40	30 min.	350	P-3.86+1.26 log t	57.6± 7.8	1.98	1.05
Control	40	—	—	P=1.60+2.21 log t	12.0± 5.0	—	—
Experiment	50	4 hours	300	P=2.18+1.74 log t	14.0± 4.9	1.16	0.79
Control	30	—	—	P=3.30+1.12 log t	28.0± 7.2	—	—
Experiment	40	1 day	300	P=3.44+0.96 log t	27.4± 7.0	0.99	0.85
Control*	20	—	—	P=1.60+2.50 log t	18.4± 7.2	—	—
Experiment*	20	2 days	300	P=2.40+1.88 log t	23.6± 9.6	1.23	0.75
Control	30	—	—	P=2.40+2.40 log t	42.0± 9.0	—	—
Experiment	40	5 days	300	P=3.90+0.98 log t	45.2± 7.8	1.06	0.41

* Centrifuging during the second half of the day.

TABLE 2

INFLUENCE OF TSISTAFOS ON TOLERANCE OF OVERLOADS BY MICE (FEMALES) 30 MINUTES AFTER ADMINISTRATION OF THE PREPARATION

Group	Dose, mg/kg	Number of animals	$P=P_0+S\cdot\log t$	Mortality (in %) at t = 10	A/A_0	S/S_0
Control	—	20	P=3.23+1.45 log t	37.4±11.1	—	—
Experiment	100	20	P=2.14+1.80 log t	14.4± 7.8	0.38	1.24
Experiment	200	20	P=1.80+2.01 log t	11.6± 7.2	0.31	1.37
Experiment	300	20	P=3.30+0.97 log t	23.2± 9.6	0.62	0.67
Control	—	40	P=2.08+1.85 log t	47.2± 7.8	—	—
Experiment	250	40	P=2.72+1.80 log t	31.2± 7.5	0.66	0.97
Control	—	20	P=2.30+1.89 log t	21.0± 9.3	—	—
Experiment	350	20	P=1.89+2.73 log t	35.2±11.0	1.67	1.44
Experiment	450	20	P=3.30+2.02 log t	62.4±11.2	2.92	1.07

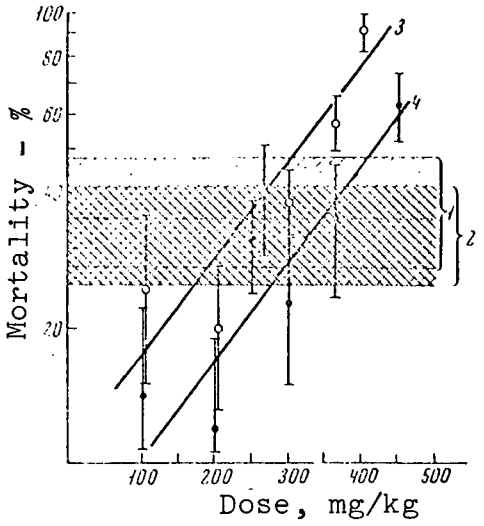

Figure 1. Influence of various doses of tsistafos on the tolerance of overloads by mice:
1 — control, females;
2 — control, males;
3 — experiment, males;
4 — experiment, females.
Confidence intervals at p = 0.05

where A is the death of animals with 10-minute centrifuging, $A = A_0$ with $D = 0$; D is the preparation dose (in mg/kg).

Figure 1 shows the dependence of the ability to withstand overloads on the dose of tsistafos. On the ordinate (logarithmic scale) we have plotted the percentage of mortality of animals with centrifuging for 10 minutes (interpolated value).

Using the method of least squares, we determined the coefficients of the resistance regression lines of the animals with centrifuging from the tsistafos dose.

Thus, in the case of the males the equation of regression will have the form: /28

$$\log A_{\sigma} = 1.005 + 0.00221 \cdot D.$$

and for females

$$\log A_{♀} = 0.785 + 0.00221 \cdot D.$$

On the basis of these equations, let us determine the tolerable dose of tsistafos. The percentage of mortality of the control animals, combined into one single sampling for the males, amounted to 33.2±4.1 (25.2-41.2, p = 0.05, n = 130 animals) and for the females 38.1±5.4 (27.5-48.7, p = 0.05, n = 80 animals).

As in the case with cystamine, let us determine the tolerable doses of tsistafos and their ranges for mice, both males and females, i.e., for A♂ = 33.2 (25.2-412) and A♀ = 38.1 (27.5-48.7), respectively.

The tolerable dose for the males was 235 (182-275) mg/kg, while for the females it was 360 (294-406) mg/kg.

Hence, the tolerable dose of tsistafos for the males was 3.7 (and for the females, 6.7) times greater than for cystamine. However, if we convert the tolerable dose of tsistafos and cystamine to the quantity of potential thiol, the tsistafos is only 1.6 times (for the males) milder than the cystamine (Table 3).

Earlier, we assumed that resistance to overloads apparently depends on the number of free mercapto groups. In particular, as we can see from Table 3, the tolerable dose as calculated for the mercapto groups in AET is slightly less than for cystamine. This possibly is related to the fact that AET does not have any free sulfhydryl groups.

If the ability of the animals to withstand overloads under the influence of cystamine and tsistafos depended only on the potentials of the mercapto groups, the tolerable doses calculated from them in these two radioprotectors would be the same. However, as we can see from the table, the tolerable doses calculated on the basis of SH-groups in cystamine and tsistafos are different. The tolerable doses calculated on the basis of the mercapto groups of AET, cystamine and tsistafos correlate to a greater degree with the toxic effect (LD_{50}) of these preparations.

TABLE 3

COMPARATIVE CHARACTERISTICS OF TOLERANCE OF OVERLOADS AND TOXICITY OF AET, CYSTAMINE AND TSISTAFOS

Preparation	Equation of overload tolerance regression	D_{tol} (mg/kg) at $A = A_{control}$	D_{tol} based on potential thiol, mM/kg	Toxicity LD_{50}, mg/kg	LD_{50} based on potential thiol, mM/kg
AET	$A=A_0+b \cdot \log D$	74* (50 — 105)	0.62 (0.42 — 0.89)	190* (182 — 199)	1.60 (1.53 — 1.67)
Cystamine	$A=A_0+b \cdot \log D$	63* (57 — 69)	0.83 (0.75 — 0.91)	244* (236 — 252)	3.20 (3.10 — 3.32)
Tsistafos	$\log A=\log A_0+bD$	235** (182 — 275)	1.31 (1.02 — 1.56)	968** (913 — 1026)	5.35 (5.10 — 5.73)

Note: D_{tol} is the tolerable dose at $A=A_{control}$, where A is mortality (in %) in the experiment, $A_{control}$ is mortality (in %) in the controls.

* For the base.

** For anhydrous salt.

Evidently the influence of radioprotectors from the group of aminothiols on the resistance of animals to overloads depends not only on the absolute content of mercapto groups, but also on other characteristics of the compound.

In contrast to cystamine, in the case of monosodium salt of β-aminoethylthiophosphoric acid, the liberation of the active mercapto group takes place only after its enzymatic hydrolysis under the influence of erythrocytes in the blood [3]. We can expect that the dependence of the effect (ability to withstand overloads) on the dose of preparation will differ from that in cystamine. As a matter of fact, in the case of tsistafos the tolerance deteriorates on a logarithmic scale, and the dose — only on a linear one. In the case of cystamine, there are reverse relationships (compare the equations of regression for cystamine and tsistafos in Table 3).

Four hours and 1 - 5 days after injection of tsistafos at a rate of 300 mg/kg, the mortality of the animals during centrifuging differed insignificantly from death among the control group (see Table 1). As shown earlier, the ability of the animals to withstand overloads under the influence of radioprotectors (cystamine, AET) is not correlated with the functional state of the heart [2].

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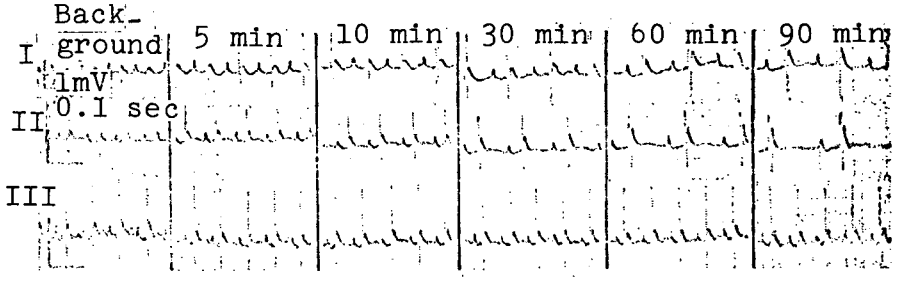

Figure 2. Changes in the EKG in mice following administration of cystamine at a dose of 150 mg/kg (I) and tsistafos at a dose of 350 mg/kg (II); III — control.

TABLE 4

CHANGES IN THE FREQUENCY OF CARDIAC CONTRACTIONS (C) AND RESPIRATION (R) IN MICE FOLLOWING ADMINISTRATIONS OF CYSTAMINE AND TSISTAFOS

Preparation	Index	Original value	Time following administration of preparation, minutes							
			10		30		60		90	
			abs	%	abs	%	abs	%	abs	%
Control	C	780	726	93	780	100	780	100	726	93
	R	270	260	97	270	100	265	99	260	97
Cystamine	C	675	580	86	485	72	450	66	384	57
150 mg/kg	R	216	104	53	174	80	156	72	150	70
Tsistafos	C	750	720	96	640	86	585	78	567	75
250 mg/kg	R	270	245	91	180	67	190	70	210	78
Tsistafos	C	702	630	90	465	66	372	53	300	43
350 mg/kg	R	240	210	88	192	80	165	69	150	63

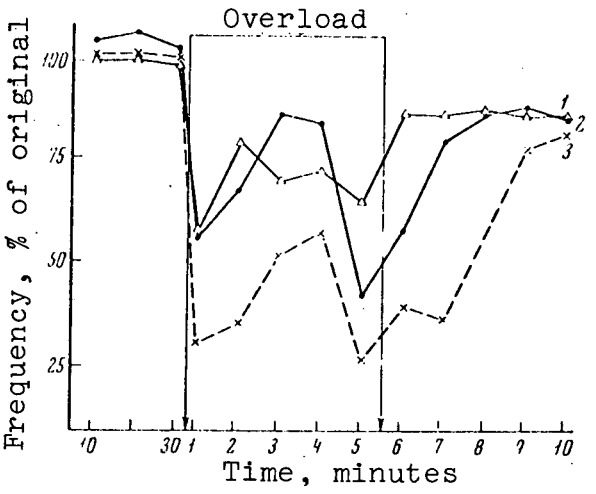

Figure 3. Changes in the frequency of cardiac contractions in guinea pigs during centrifuging under the influence of tsistafos at a dose of 100 mg/kg (2) and 200 mg/kg (1). 3 — control.

As a matter of fact, as we can see from Table 4 the frequency of the cardiac contractions and the respiration frequency under the influence of cystamine and tsistafos decrease, although at the same time there is a normalization of the reactivity of the organism with respect to overloads. The qualitative changes in the ECG under the influence of tsistafos at a dose of 350 mg/kg were the same as for cystamine at a dose of 150 mg/kg (Figure 2).

In experiments on guinea pigs, we studied the reaction of the heart to overloads following injection of tsistafos. Thirty minutes prior to centrifuging, the animals received intraperitoneal injections of tsistafos in doses of 100 and 200 mg/kg. The animals were centrifuged for 5 minutes at an overload value of 22 units, and the ECG was recorded with three standard leads. Among the control guinea pigs, during the first minute of centrifuging, there was an acute bradycardia: the frequency of the cardiac contractions fell to 72%. During the fourth minute of centrifuging, the frequency of the cardiac contractions normalized somewhat (58% of the original level), but by the end of rotation there was a second wave of acute

bradycardia. By the 4^{th} to 5^{th} minutes after rotation, the pulse frequency was 20% lower than the original level.

Under the influence of tsistafos, the nature of the changes in the frequency of cardiac contractions was the same as for the control guinea pigs, but less pronounced, especially at a dose of 200 mg/kg. The initial bradycardia in the experimental animals under the influence of overloads was less: the frequency of cardiac contractions fell only by 45% (Figure 3). Restoration of the frequency of the cardiac contractions following rotation began 1 to 2 minutes faster than in the control guinea pigs. Figure 4 shows the ECG under the influence of overloads in the control animals and following injection of 100 and 200 mg/kg of tsistafos. Then no characteristic differences in the ECGs of the control and experimental animals were observed.

Hence, drawing a brief conclusion, we can say that first of all tsistafos has a different effect on the ability of animals to withstand overloads than does cystamine. The dose effects with respect to cystamine and tsistafos are described, respectively, by different regression equations: /32

$$A = A_0 + b \cdot \log D \quad \text{and} \quad \log A = \log A_0 + b \cdot D.$$

Tsistafos, in comparison to cystamine, has a less pronounced effect on the ability of animals to withstand overloads.

In the second place, the doses of AET, cystamine and tsistafos which can be withstood (on the basis of the overload resistance criterion), when converted to potential mercapto groups, are correlated with their toxic effect (on the basis of LD_{50}).

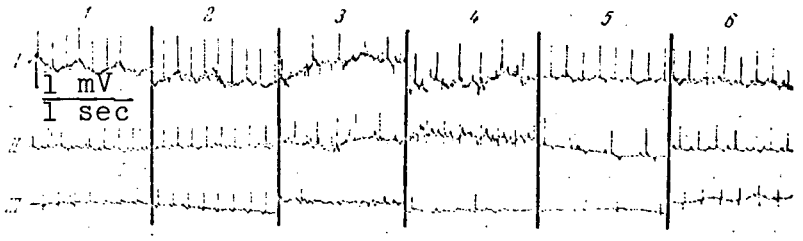

Figure 4. Changes in the EKG in guinea pigs under the influence of an overload (22 units, 5 minutes) following preliminary administration of tsistafos at a dose of 200 mg/kg (I) and 100 mg/kg (II). III - control; 1 - background; 2 - 30 minutes after administration of tsistafos (prior to centrifuging); 3 - 1 and; 4 - five minutes of rotation; 5 - 1 and 6 - five minutes following rotation.

In the third place, the tolerable doses of aminothiols which we investigated were 2.6 (for AET) and 4 (cystamine and tsistafos) times less than their toxic effect (on the basis of LD_{50}).

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Influence of Radioprotectors from the Aminothiol Group on the Cardiac Function of Guinea Pigs Under the Influence of Overloads

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V. A. Kozlov and B. I. Davydov

ABSTRACT. Cystamine, mercaptopropylamine and AET reduce the resistance of guinea pigs to overloads. The bradycardia they produce probably is intensified by the similar action of overloads alone and accelerates the exhaustion of the adaptation reserves during their action. AET did not affect the frequency of cardiac contractions, but under the influence of acceleration acute bradycardia and subsequent death of the animals also ensued. The influence of cystamine on heart function may be completely or partially eliminated with the aid of atropine or dimedrol. However, normalization of the resistance of the organism to overloads could not be achieved in this fashion.

Regardless of the fact that at the present time the pharmacological effects of radioprotectors have been sufficiently well-investigated, the reactions which arise under their influence in conjunction with dynamic spaceflight factors may be different. As we know, under the influence of radioprotective preparations (cystamine, AET, serotinin), the resistance of the organism to dynamic factors decreases [4]. In this connection, we undertook a more detailed study of the influence of radioprotectors, in particular, the function of the cardiac system under overload conditions, inasmuch as it is known that the ability to withstand transverse accelerations is primarily

CHANGES IN THE FREQUENCY OF CARDIAC CONTRACTIONS IN GUINEA PIGS (% OF ORIGINAL VALUE) FOLLOWING ADMINISTRATION OF CYSTAMINE AND AET

Preparation	Dose, mg/kg	Time following administration of preparation, min.				
		1 — 3	5	10	15	30
Cystamine	45	125	120	114	134	165
	100	95 — 105	92 — 110	95 — 110	102	109
	125	96	79	61	57	53
	150	50 — 77	45 — 82	42 — 77	50 — 77	47 — 51
AET	100	100	100	97	97	100
	150	101	95	95	105	98

determined by the compensatory capacity of this system [2, 3]. A study of heart function was performed using the most effective and promising radioprotectors.

The experiments were performed on 50 guinea pigs of both sexes, weighing 350 - 500 grams. Thirty to 60 minutes prior to centrifuging, the animals were given cystamine (45 - 150 mg/kg), β-mercaptopropylamine (MPA) (150 mg/kg) and AET (100 - 150 mg/kg). The preparations were dissolved in distilled water and injected in a volume of 0.5 ml intraperitoneally, directly in the centrifuge compartment. The animals were not held down. They were centrifuged twice with a five-minute interval between. The magnitude of the overload was 22 units (0.7 units per second); the direction was from the back to the chest.

In all experiments, we recorded the electrocardiograms with three standard leads: two animals were centrifuged

simultaneously - an experimental one (which had been injected with the radioprotector) and a control one. The ECG was recorded for 5 minutes prior to centrifuging or injection of the preparations, and then every 1 to 2 minutes following their injection and continuously during the centrifuging process.

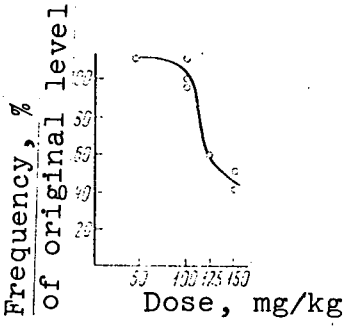

Figure 1. Changes in the frequency of cardiac contractions in guinea pigs as a function of cystamine dose.

In the EKG, we analyzed primarily the RR interval (frequency of cardiac contractions) and the changes in the QRS complex.

Studies showed that cystamine (150 mg/kg) and β-mercaptopropylamine (150 mg/kg) have a sharply pronounced influence on the rhythm of cardiac activity. In all experiments, after injection of the preparations, there was a pronounced slowing of the cardiac rhythm. The frequency of the cardiac contractions, only 1 to 3 minutes after injection, had fallen usually by 23 to 50% (see the table).

Normal cardiac rhythm was restored 2.5 to 3 hours or more following injection of the radioprotectors. There were no significant differences in the effect of cystamine and β-mercaptopropylamine at these doses on the function of the heart. It is interesting that cystamine at a dose of 45 mg/kg /34 had a strengthening effect on the frequency of the cardiac contractions. Figure 1 shows the data on changes of the cardiac contraction frequency as a function of the cystamine dose. One can readily see the S-shaped dependence of the effect on the dose. In studying AET (100 - 150 mg/kg), we did not see any slowing of the rhythm of cardiac contraction.

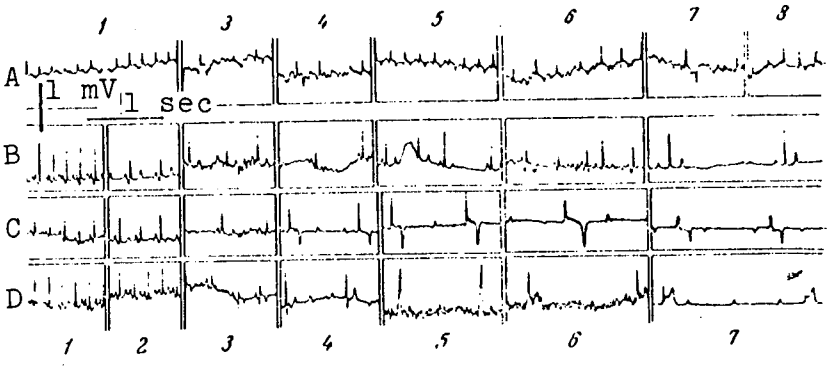

Figure 2. EKG of guinea pigs subjected to the action of transversely directed overloads (22 units) in control experiments (A), following preliminary administration of cystamine (B), β-mercaptopropylamine (C) and AET (D). 1 - background; 2 - fifteen minutes after administration of protectors; 3 and 4 - at first and fifth minutes of first overload; 5 - five minutes following first overload; 6 and 7 - first and fifth minutes of second overload; 8 - five minutes after end of second rotation.

The differences which were found in the reaction between cystamine and MPA, on the one hand, and AET, on the other are interesting from the standpoint that these preparations belong to the same group of chemical compounds, the mercaptoalkylamines. It should also be mentioned that according to the data in the literature there are also differences between these preparations regarding the reaction involving blood pressure: cystamine, MPA and tsistafos cause a hypotonic effect in various animals, while AET has a hypertonic effect [5].

In a second series of experiments, we studied the influence of cystamine, MPA, and AET on cardiac function when the organism was exposed to overloads that were critical in magnitude.

The control animals, which had not received radioprotective preparations, reacted to an overload of 22 units by a decrease in the frequency of the cardiac contractions. After cessation of the overloads, there was a period of recovery when the frequency of the cardiac contractions returned to the original level or slightly below.

A second exposure to overloads was characterized by the same changes, and after it had ceased there was a period of complete recovery of the heart function. The mortality of the control animals following a double centrifuging of this kind was 30 - 40%.

The reaction of the animals which had received radioprotective preparations was generally the same for all of the preparations tested, and was characterized by a more rapid and pronounced (as compared to the control guinea pigs) development of decompensation of the cardiac activity. A sharp decrease in the frequency of cardiac contractions was observed only one minute following the start of centrifuging. The period of recovery of cardiac activity following the termination of the first centrifuging either was not observed at all, or was insignificant.

All of the guinea pigs, following injection of cystamine, MPA, and AET, died after the first or second centrifuging. The general nature of the changes in the frequency of the cardiac contractions following injection of these preparations is illustrated in Figure 2.

Hence, the experiments on guinea pigs showed that cystamine, MPA and AET reduce the resistance of the animals to withstand overloads. Cystamine and MPA in guinea pigs produced bradycardia,

which probably becomes stronger with similar action of the overloads themselves, and accelerates exhaustion of the adaptation reserves under the influence of overloads. Although AET does not influence the frequency of cardiac contractions, under the influence of acceleration there was also a sharp bradycardia and subsequent death of the animals. Most likely, it does not /36
follow from this that the influence of the radioprotectors on the heart function, or in other words, the state of the cardiac function prior to centrifuging, cannot play a specific role in the ability of the organism to withstand accelerations. It was clear, however, that the original (prior to centrifuging) functional state of the cardiac activity is not the principal and decisive factor in the mechanism of this phenomenon.

The similarity in the action of the tested preparations on the heart function under overload conditions may serve as an indication that they reduce the tolerance to overloads. On the other hand, this action, taking into account the differences in the influence on the function of the heart, is apparently caused by effects other than an influence on heart rhythm.

To test this assumption, we performed special experiments on guinea pigs in which we eliminated or reduced the bradycardia effect of cystamine with the aid of pharmacological substances, or (on the other hand) produced bradycardia (experiments with AET). For this purpose, we tested substances with various pharmacological effects on the function of the cardiovascular system — cholinolytic (atropine), cholinergic (pilocarpine), adrenergic (adrenaline) and dimedrol. We know that the latter substance, like a number of other antihistaminic preparations — at the same time that it blocks many effects of histamine — also has a pronounced anticholinergic effect and intensifies some of the effects of adrenalin [1].

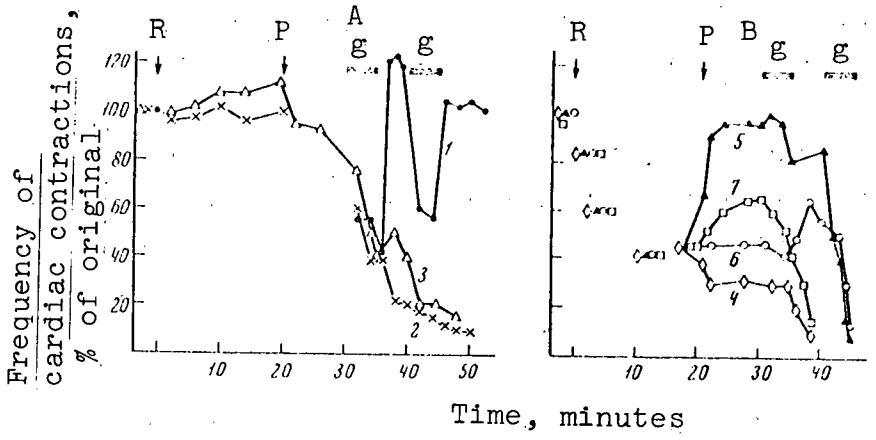

Figure 3. Influence of pilocarpine, atropine, adrenalin and dimedrol on the frequency of cardiac contractions under the influence of overloads against the background of AET (A) and cystamine (B).
1 - control; 2 - AET; 3 - AET + pilocarpine; 4 - cystamine + pilocarpine; 5 - cystamine + atropine, 6 - cystamine + adrenalin; 7 - cystamine + dimedrol; R - moment at which radioprotective preparation is introduced; P - moment at which pilocarpine, atropine, adrenalin or dimedrol are introduced; g - action of overload.

The order of the experiments was as follows: at the beginning of the experiment, we recorded the normal electrocardiogram, then made a recording after injecting the radioprotector. Then the substance to be investigated was administered and the ECG recorded once again, in order to determine the changes in the cardiac activity under the influence of this substance. Then the animals were centrifuged, with the ECG recorded simultaneously. The test substances were administered intraperitoneally in previously selected doses (atropine, 2 mg/kg, pilocarpine, 2 mg/kg, adrenalin, 0.25 mg/kg and dimedrol, 20 mg/kg). In these quantities, these substances themselves did not have a significant influence on the ability of guinea pigs to withstand overloads.

The results of the experiments showed that atropine completely (and dimedrol - partially) reduces the parasympathotonic (relative to the frequency of cardiac contractions) effect of cystamine and MEA on the heart. Adrenalin (at the tested dose) does not prevent this effect; when it is used against a background of antiradiation substances, we observed only an increase in the voltage of the R peaks. Against a background of cystamine and AET, pilocarpine produced its usual effect.

Hence, the influence of cystamine on the heart function /37
may be eliminated completely or partially by means of atropine and dimedrol. However, as the results of experiments have shown (Figure 3), the ability of animals to withstand overloads remained low and corresponded roughly to the level which was observed when guinea pigs were given individual radioprotectors. By removing one of the most important effects of radioprotectors, the influence on the heart function, it is impossible to normalize the resistance of the organism to overloads.

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The Mechanism of Reduced Ability to Withstand Overloads Under the Influence of Radioprotective Pharmacological Substances

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ABSTRACT. The rats were subjected to the action of transverse overloads (30 units, 5 minutes), cystamine (75 mg/kg) or combined effect of these factors. The authors studied the pathomorphological and histochemical changes in the lungs, liver heart, diaphragm and adrenals. The action of overloads or the administration of cystamine created a state of oxygen starvation of the tissues, and rapid consumption and exhaustion of energy reserves in the organism of the animals. Under the combined influence of cystamine and overloads, the effect of hypoxia was cumulative.

Certain radioprotective substances, such as cystamine and /38
aminoethylisothiuronium, reduce the ability to withstand overloads [5]. Discovery of the mechanisms involved in this phenomenon is important for determining ways of increasing the resistance of the organism during extremal influences using radioprotectors.

The purpose of the investigation is to study the pathomorphological and histochemical changes in the organs of the animals under the influence of overloads, cystamine, and its compounds

for the purpose of clarifying possible reasons for the intensifying effect of these two factors.

The experiments were performed on 60 random-bred white rats of both sexes, weighing 180 to 200 grams. Twenty animals were subjected to overloads, 20 received cystamine and 12 were subjected to the joint effect of overloads and cystamine; 8 rats served as a biological control. The magnitude of the overloads was 30 units, lasting 5 minutes, applied from the chest to the back. Cystamine dichlorohydrate was administered intraperitoneally in a dose of 75 mg/kg; in the combined application, the preparation was administered 15 minutes prior to centrifuging. The rats were sacrificed 30 minutes, 6 hours, 1 and 3 days after exposure to these effects. Sections of the liver, heart, diaphragm and adrenals were prepared from combined tissue blocks freshly frozen in dry ice, and the fat content was determined by means of scarlet red, glycogen according to Shabadash, RNA concentration according to Brache, using methyl green — pyronine, alkaline phosphatase activity according to Homori, succinate dehydrogenase according to Nachlass and others, and monoamino-oxidase according to Glenner [20]. The intensity of the histochemical reactions was evaluated on a five-point scale.

Under the influence of the overloads, 7 rats died (35 $\pm$ 11.3%); 9 died under the combined influence of cystamine and overloads (75 $\pm$ 13.5%); among those animals which received cystamine, there were no deaths. In those animals which died, the dorsal portions of the lungs showed zones of partial collapse and acute hyperemia, sometimes extending as far as the radical segments of the lungs. The ventral portions of the lung tissue, on the other hand, were pale, and lacked blood. In those animals which were killed, zones of hyperemia were absent; all portions of the lungs showed uniform and numerous hemorrhages, from minor

to major, occupying the entire area; the arteriovenous anastomoses were wide open.

Following exposure to the overloads, the liver and myocardium of the animals initially showed a drop in glycogen, which was restored after a few hours, and after 3 days was higher than the original value (Table 1). In the diaphragm, the content

TABLE 1

Time after influence, hours	Liver				Myocardium				Diaphragm			
	1	2	3	4	1	2	3	4	1	2	3	4
0.5	↓↓↓	↓	↑	↑↑	↓↓↓	↓↑	↑	↓↑	↓↓↓	↓↓	↓↑	↓↑
6	↓↓	↑	↑	↑	↓↓	↓↑	↑	↓↑	↓↓↓	↑	↓↑	↑
24	↓↑	↓↑	↓↑	↓	↓↑	↓↑	↓↑	↓↑	↓↓↓	↓↑	↓↑	
72	↓↑	↓↑	↓↑	↓↑	↓↑	↑	↓↑	↓	↓	↓↑		↓↑

Note: 1 — Content of glycogen; activity; 2 — succino-dehydrogenase; 3 — alkaline phosphatase; 4 — monoaminooxidase; ↓ — decrease relative to original; ↑ — increase; ↓↑ — unchanged; none — no investigation performed. The number of crossbars indicates the degree of change.

of glycogen was reduced not only during the initial period, but also 3 days later, which may indicate a continuing forced operation of this "second heart" [7] for a long time, even when no longer subjected to this influence. /39 The change in activity of succino-dehydrogenase was most clearly evident in the liver. After brief suppression, the activity of the succino-dehydrogenase increased after 6 hours and remained at high level until the end of the observations. In the heart and diaphragm, the activity of this enzyme did not change. Alkaline phosphatase

and monoaminooxidase in the liver increased during the first few hours and returned to the control level after 1 to 3 days. In the heart and diaphragm muscles, the activity of these enzymes changed slightly. If we compare the changes in the glycogen content and enzymatic activity — which take place in the liver following exposure to overloads — with the changes in this organ which are observed following injection of cystamine, we can see a certain similarity (Table 2, Figure). When cystamine is administered, as when overloads are applied, there are several phases in these changes. The content of glycogen dropped sharply

TABLE 2

Time after introduction, hours	Lipids	Glycogen	Succino-dehydrogenase	Alkaline phosphatase	RNA
0.5	↓	↓	↓	↓	↓↑
6	↓	↓	↓	↓	↓
24	↓	↑	↓	↓	↓
72	↓	↓	↓	↓	↓↑

Note: Symbols same as in Table 1.

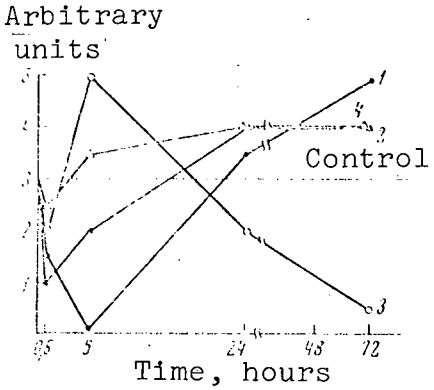

Figure. Glycogen content in the rat liver after administration of cystamine (1) and after action of overload (2). The activity of succino-dehydrogenase following administration of cystamine (3) and following action of an overload (4)

after 30 minutes and (especially after 6 hours) returned in several days and was higher than the control after 3 days. The activity of succinate dehydrogenase and alkaline phosphatase, after a slight decline, returned to normal after 6 hours and fell during subsequent periods. In addition, there were also differences which consisted in a more pronounced decline in the glycogen content and the enzymatic activity when cystamine was administered. The reason for these differences apparently has to do with the recovery of disturbed homeostasis following the overload. According to the data of Bacq et al.[23], cystamine is found in the liver 24 hours after its injection into the organism. /4

In those animals which survived following combined exposure to cystamine and overloads, these changes were the same as with exposure to each of these two factors individually, but were more pronounced. The content of glycogen had not recovered by the third day. Immediately after exposure the activity of succinate dehydrogenase dropped sharply and recovered only after a few days. The decrease in the activity of alkaline phosphatase was slight, being observed only after 30 minutes, while during subsequent periods the activity of the enzyme was increased.

The changes in the adrenals under the influence of overloads or cystamine also were similar, especially during the first few hours, and were characterized by a decline in the lipid content, an increase in the RNA concentration in cells in various zones of the gland, an increase in the activity of succinate dehydrogenase and a decline in alkaline phosphatase activity (Table 3).

The hypostases observed in those animals which died (in the lungs) indicate a pronounced and stable redistribution of the blood in the vessels of the lung tissue as well as a shifting of the blood in the direction of the acting centrifugal force. The

same redistribution of the blood in the lungs under the influence of overloads was observed by Muller [31], V. M. Klebanov et al. [9] and in x-ray studies of the chest made directly during the application of overloads by Fisher [28], Beckman et al. [25] and A. R. Mansurov [15].

TABLE 3

Periods after influence, hours	Zones of the adrenals											
	Glomerular				Fascicular				Reticular			
	1	2	3	4	1	2	3	4	1	2	3	4
	After overload											
0.5	↓	↓↑	↓↑	↓↑	↓	↓↑	↓↑	↑	↓	↑	↓↑	↓↑
6	↓	↓↑	↓↑	↓↑	∓	↑	↑	↕	∓	↕	↑	↕
24	↕	↑	↓↑	↓↑	∓	↕	↑	↕	↕	↕	↑	↕
72	↓	↓↑	↓↑	↓↑	↕	↑	↓↑	↑	↓	↑	↓↑	↑
	Following administration of cystamine											
0.5	↓	↓↑	↓	↓↑	↓	↓	↓↑	↓	↓	↓	↓	↓
6	∓	↕	↕	↕	↓	↓↑	↕	↓↑	∓	↑	↓	↑
24	↓↑	↑	↕	↑	↓	↓↑	↓	↓↑	↕	↓	↓	↓
72	↓↑	↓↑	↕	↓↑	↓↑	↓↑	↕	↓↑	↓↑	↑	↑	↑

Note: 1 — fat content; activity; 2 — succinodehydrogenase; 3 — alkaline phosphatase; 4 — concentration of RNA. For the other symbols, see Table 1.

Transverse overloads of significant magnitude reduce the depth of respiration, the chest is compressed and the area of the pulmonary field is reduced [1]. Gauer [29] showed that during the action of the maximum tolerable overloads, the minute volume of the loads decreases and the vital capacity of the

lungs decreases due to the compression of the chest cavity. Since under these conditions there is an intensification of breathing by means of the diaphragm [26], the retention of the respiratory function during overloads depends to a considerable extent on the reliability of its operation.

Increased work by the diaphragm in our experiments was indicated by the fact that it showed a sharp decline in glycogen content. It is characteristic that there was a particularly sharp drop in the glycogen content in the diaphragm of the animals which had died.

The change in lung hemodynamics leads to disruption of the normal ratio of blood and air and to arterial pulmonary shunting, a drop in the volumetric content of oxygen and saturation of the arterial blood with oxygen [8].

Consequently, one of the mechanisms for the unfavorable action of overloads on animals is disruption of the respiratory function and attenuation of the oxygenation of the blood in the lungs.

Another reason for the development of acute hypoxia in the death of rats, as indicated by the investigations of many authors, is a deterioration of the operating conditions of the heart, associated with a sharp increase in the hydrostatic pressure of the blood [28, 32]. /42

Hypoxia associated with disruption of respiration and cardiac activity increases still more due to inversion of circulation in all organs [19]. The tissues and organs suffer from a shortage of oxygen because the regional blood circulation changes. Hall and Granmo [30], by means of rapid freezing of

animals during centrifuging, showed that (for example) the shape of the liver changes, one half becoming anemic and the other filled with blood, and stagnant.

Acute hypoxia of a mixed variety sharply disturbs the metabolism in the tissues. Tissue respiration declines [16]. Non-oxidized products accumulate in the organism; the pH of the blood becomes acid [11]. As a result of all these changes, there is a marked disruption of the energy production required both for the vitally important organs (including the contractile activity of the myocardium and the maintenance of vascular tone) and for the function of respiratory muscles, especially the diaphragm.

In the animal organism, the principal and most mobile original energy source whose oxidation during respiration insures accumulation of high-energy phosphate bonds of adenosine triphosphoric acid (ATP) and creatine phosphate (CP) is glycogen [21, 22]. In those animals which survived, as a result of the stressed activity of the cardiovascular system, glycogen vanished from the myocardium, diaphragm muscle, and also from the principal deposit — the liver cells.

In those animals which died during exposure to overloads, due to local stagnation of the blood, glycogen was unable to leave the liver to enter the general circulation and to reach the working organs. Therefore, glycogen was found in the liver in significant amounts, while it had disappeared from the muscles of the heart and diaphragm.

The disappearance of glycogen from the tissues is still further accelerated by a shortage of oxygen, i.e., in hypoxia, during which there is a predominance of anaerobic glycolytic processes which are not economical from the energy standpoint.

This is indicated by a decline in the activity of succino-dehydrogenase immediately following the action of overloads. An increase in the activity of succinate dehydrogenase after 6 hours and several days was caused by the restoration of the original energy balance and increased oxidation of the accumulated non-oxidized products, and is supported by increased oxygen consumption. As indicated by the studies of V. V. Matsynin [16], under the influence of overloads the consumption of oxygen by tissues increases subsequently, i.e., this is nothing more than the so-called "liquidation of the oxygen debt".

An important role in the maintenance of the necessary vascular tone and in the regulation of cardiac activity under the influence of overloads on the organism is played by the adrenal hormones [2, 18]. The histochemical changes which we observed in /43
the adrenals of rats subjected to the action of overloads indicate an increase in the activity of this gland. In the decompensation of blood circulation under the combined influence of cystamine and overloads, we cannot deny the disruption of the correlative function of the central nervous system [3].

In all of the surviving animals, 30 minutes after exposure to overloads, the liver showed a marked increase in the quantity of cells containing fatty inclusions. In the animals which died, fatty dystrophy was less pronounced. Consequently, the contents of fat and glycogen in the liver bore an inverse relationship to each other. The existence of such inverse relationships between the contents of glycogen and lipids in the liver is explained by the close relationships between carbohydrate and fat metabolism; this combination occurs in particular at the stage of triosophosphates or through subsequent β-oxidation of fatty acids to form pyruvic acid [10, 17]. Consumption of glycogen leads to mobilization of fats from the fat deposits [13]. This is indicated by the fact that under stress there is a sharp increase in

the activity of lipolytic enzymes in the fat deposits [12]. Fat in the form of incompletely substituted esters of glycerine or in the form of free fatty acids passes through the blood into the liver. Its oxidation in the liver cells is complicated due to the fact that, under the influence of overloads, there is essentially anaerobic glycolysis with accumulation of non-oxidized products, indicated by an increased activity of monoaminooxidase. Therefore, the reaction shifts toward the synthesis of esters of glycerine with fatty acids, i.e., toward the formation of neutral fats [14] and the accumulation of the latter in the liver cells.

When the animals are given certain protectors (cystamine, AET, etc.), a state of hypoxia develops in the organism [4, 27]. This explains the unique pattern of histochemical changes which we found both under the influence of overloads and when cystamine was administered. The results obtained are supported by other studies [6, 23].

Hence, both the effect of overloads and the administration of cystamine to animals produce in the organism a state of oxygen starvation of the tissues which is the same in terms of its final outcome, as well as a rapid breakdown and depletion of energy supplies. Under the combined influence of preliminary administration of cystamine and overloads, this effect is cumulative. Administration of cystamine in addition not only reduces the content of the energy material in the most important organs, but during the action of the overloads apparently also has an inhibiting effect on the processes of energy formation, especially the aerobic processes. Therefore, the deaths of the animals increase under the combined influence of overloads and cystamine.

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Influence of Certain Radioprotectors on the Ability of White Rats to Withstand Acute Hypoxia

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ABSTRACT. In experiments on white rats, the authors studied the nature of the response reaction of an organism protected by means of radioprotectors to the action of acute hypoxia. Cystamine (250 mg/kg), AET Br (800 and 250 mg/kg), when administered intraperitoneally and orally, create a marked decrease in the resistance of the animals to acute hypoxic hypoxia and hypoxia created by carbon monoxide.

The possible pathophysiological mechanisms of this phenomenon are discussed.

In certain cases, radioprotectors may be used when not only /45
radiation, but also other environmental factors, especially acute hypoxia, are influencing man.

In this paper, we studied the action of the most popular radioprotectors, cystamine and AET, on the resistance of white rats to acute hypoxia produced either by a drop in barometric pressure in a barochamber or by using carbon monoxide.

The material available in the literature dealing with the pharmacology and the biochemistry of cystamine and AET is highly contradictory and makes it impossible to predict without

experimental testing the reaction of the organism following administration of these radioprotectors on acute hypoxia. Thus, according to the data of L. I. Yampol'skaya [7], cystamine reduces the utilization of oxygen in rats and mice. At the same time, Bacq and others [8] did not observe the influence of this preparation on absorption of O_2, while V. I. Kuznetsova and L. I. Tank [3] observed an increase in the consumption of oxygen under the influence of cystamine. As contradictory as the information is regarding the influence of cystamine and AET on tissue respiration, A. S. Mozzhukhin and F. Yu. Rachinskiy [4] indicate that cystamine and AET do not have a great influence on the course of oxidation processes in the tissues [7, 10].

Ye. F. Romantsev [5] did not detect a decline in the activity of cytochromoxidase in the transversely striated muscles produced by cysteamine. At the same time, however, Lelivre [11, 12] states that cysteamine produces a decrease in oxygen consumption by the tissues, and causes deterioration of the mitochondria in the liver and kidneys of the rat. With respect to AET, data are available (Tsins et al.) [14] which indicate that this radioprotector inhibits the activity of cytochromoxidase and succino-dehydrogenase. These authors have shown that 30 minutes after administration of AET to the rats at a dose of 190 mg/kg, homogenates of the liver and kidneys show an inhibition of oxidation of pyruvate and α-ketoglutorate. Ilvan [13] presents data indicating inhibition of brain respiration by thiols.

A. G. Sverdlov et al. [6] studied the influence of certain radioprotectors on the change in pO_2 in the spleen and thigh muscles in mice using the polarographic method. The analysis of the changes which were observed revealed that in 1/3 of the

cases cystamine resulted in a significant (13%) increase in pO_2 in the spleen, while in the others there was a pronounced hypoxic effect, reducing pO_2 by 18%. Approximately the same contradictory data were obtained when AET was administered.

Hence, the analysis of the data in the literature leads to the necessity of an experimental investigation of the responsive reaction of "protected" organisms to the action of acute hypoxia. The experiments were performed on male and female white rats weighing 180 - 200 grams. Radioprotectors were administered /47 intraperitoneally to the animals 10 to 15 minutes prior to exposure to the influence of acute hypoxia. The experimental and control animals were placed simultaneously in a barochamber with a capacity of 0.035 m^3, where they were kept for 12 minutes at a residual pressure of 160 mm Hg ("altitude" of 11,368 m). The data on the influence of cystamine and AET on the ability of the animals to withstand acute hypoxia are shown in Table 1. Similar experiments were performed under the acute influence of carbon monoxide (Table 2).

Hence, cystamine and AET in the doses investigated with intraperitoneal and oral administration significantly reduced the resistance of animals with respect to hypoxia. Bacq et al. [9] showed that cystamine reduces the resistance of rats to lowered barometric pressure. As our studies showed, this effect is also characteristic of S,β-aminoethylisothioronium (AET). They cause a decline in the resistance both with respect to hypoxic hypoxia as well as hypoxia produced by the toxic action of CO_2.

The data obtained indicated that an organism "protected" by pharmacochemical methods against the action of radiation may

TABLE 1. INFLUENCE OF CYSTAMINE AND AET ON THE RESISTANCE OF WHITE RATS TO ACUTE HYPOXIC HYPOXIA

/46

Group*	After influence of hypoxia		Group*	After influence of hypoxia	
	Dead	Survived		Dead	Survived
Cystamine, 200 mg/kg, intraperitoneally, 15 minutes prior to exposure	16	4**	AET Br, 250 mg/kg, 15 minutes prior to exposure	11	9
Control	4	16	Control	4	16

*Twenty animals per group.

** $p < 0.02$.

TABLE 2. INFLUENCE OF CYSTAMINE AND AET ON THE RESISTANCE OF WHITE RATS TO ACUTE HYPOXIA PRODUCED BY CARBON MONOXIDE

Radioprotector and dose, mg/kg	Time of injection of radioprotector prior to CO poisoning	Concentration of CO in the poisoning chamber, %	Poisoning time, minutes	Number of animals	Death following exposure
Cystamine, 250	1 hour	1	10	11	10*
0	—	1	10	11	4
AET-Br, 800	1 hour	1.5	5	10	10*
0	—	1.5	5	10	4
AET-Cl, 250	10 minutes	1.5	5	20	15*
0	—	1.5	5	20	5
AET-Br 250	5 minutes	1.0	10	10	6
0	—	1.0	10	10	3

* $p < 0.02$

be weakened with respect to the action of other environmental factors. In this connection, it becomes necessary to use antihypoxic substances when aminothiols are used as radioprotectors.

In must be pointed out in this connection that, according to certain data [2], the radioprotectors which do not belong to the aminothiol group confer increased resistance of the organism to hypoxia. Thus, 5-MOT weakens the harmful effect of acute, subacute and chronic hypoxia. Similar data were obtained when using antioxidants — propylgallate, ionol, ambunol [1].

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II.

INFLUENCE OF FLIGHT FACTORS ON THE REACTIVITY OF ANIMALS TO MEDICINAL SUBSTANCES

Certain Aspects of the Practical Utilization of Medicinal Substances Under Flight Conditions

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ABSTRACT. The article presents some ideas concerning the indications and contra-indications of certain medicinal substances by the crew of aircraft during flight. A brief summary of the characteristics of medical illness is presented.

Restoration of disrupted physiological functions in cosmonauts and pilots or the prevention of these disruptions is accomplished by a number of measures, which include the rational utilization of various pharmacological substances. On space-flights, especially those over long distances or lasting long periods of time, it is quite obvious that a necessity might develop for using medicinal substances both for prophylaxis (for example, radiation sickness, neuro-psychic or infectious diseases) and other purposes such as the treatment of ailments which may develop.

On long spaceflights, the complex action of several factors may promote the development of a different type of reactive state in the cosmonauts, such as emotional (psychic) stress, going far beyond the limits of the physiological capacity of the organism. This type of condition unavoidably has a negative effect on working ability and tolerance of the organism. Current, and particularly future, aviation techniques also make serious demands on the moral, psychic and physiological qualities of the pilot.

Consequently, seeking and developing effective measures and methods directed both at retention of working ability and increasing the resistance of the organism to the unfavorable effect of extremal flight factors is one of the most important problems in aviation and space medicine. Among the methods which may be /49
used for increasing the resistance of the organism to flight factors, an important (we might even say critical) one is the use of pharmocological substances.

Medicinal substances, to use the well-chosen phrase of I. P. Pavlov, are the universal weapons of the doctor. However, this weapon must be studied carefully so that one knows how to use it correctly. Otherwise, instead of the expected effect, considerable harm may be done. Due to the specific conditions of flight activity, the administration of certain medicinal preparations to crew members aboard spacecraft during flight calls for maximum care and training.

In this connection, I would like to say several words in regard to self-treatment and medicinal ailments.

During the last decade, we have seen the term "drug disease" used more and more in the literature. The term is completely justified, and drug disease really exists. Drug

disease is not poisoning by preparations as a result of overdosage or complications of pharmacotherapy. Drug disease is nothing more than a different kind of allergic reaction caused by specific sensitivity to medicinal substances.

One of the characteristic features of drug disease is the very high sensitivity of sensitized persons to small doses of certain medicines. This sort of increased sensitivity to chemical preparations does not arise immediately, but a short time after the beginning of treatment.

Experiments on animals and clinical observations have shown that certain compounds, based on a benzene ring with NH_2-groups attached to it or atoms of chlorine, have high antigenic properties, especially if these compounds contain oxygen groups (for example, sulfon or arsenic). Somewhat later, other highly-antigenic chemical substances were found (especially with a nucleus of pyrimidine). Sulfanilamide (white streptocide) is a typical example of a chemical structure with a benzene ring, to which an NH_2 group is attached, and thiouracil is an example of a chemical compound with a pyrimidine nucleus.

The sulfanilamide preparations and thiouracils cause a number of reactions involving drug intolerance. Even such an apparently harmless preparation as thiamine (Vitamin B_1) in certain people may sometimes cause serious allergic reactions, leading even to shock.

In the following, we have listed preparations which, according to the data of G. L. Aleksander, are the most dangerous and cause various types of allergy from mild rashes to shock: pyramidone, aspirin, dimedrol, antipyrine, quinine, iodides, /50
arsenic, thiamine (B_1), sulfanilamides, penicillin, streptomycin,

levomycetin (chloroamphenicol), barbiturates, Novocaine, acrichine, and cortisone.

A number of medicinal preparations which can produce sharp functional disruptions in the organism of a pilot or cosmonaut (inhibition of the central nervous system), depression of the marrow, disruption of the cardiovascular system, hypoxia, etc.) must be either completely excluded from use in aviation and space medicine or used in extreme cases and only under the supervision of a physician. These preparations include the neuroplegics ("great tranquilizers") or depressants (substances which block the nervous system). Typical representatives are aminazine, narcotics, certain antipyretics (phenacetin), vasodilators (amyl nitrite, nitroglycerine), tranquilizers ("calming substances"), for example, meprotan (andaxin), Librium (elenium), antihistaminic preparations (dimedrol, diprozin).

Such medicinal preparations as the following should not be used prior to flight (or in flight):

— if they lead in some way to oxygen insufficiency, for example, if they produce inhibition of the respiratory center, form metahemoglobin in the blood, inhibit (or block) tissue respiration enzymes;

— if they inhibit the central nervous system (barbiturates);

— if they produce markedly pronounced allergic reactions (the preparations listed above);

— if they reduce the resistance of the organism to flight factors.

We know, for example, that sulfanilamide preparations may intensify the phenomena of hypoxia in flight due to tissue anoxia.

Certain preparations, the derivatives of aniline used as sedatives (calmants), substances (phenacetin, paracetamol) from the acridine series, used as antimalarial agents (acrichine), may lead to the formation of metahemoglobin.

The altered chemical state of the blood, produced by these preparations, may reduce the compensatory and adaptive mechanisms and the resistance of the organism to the unfavorable factors of high altitude flight.

The duration of the aftereffects of all these preparations differs. It depends on the physical-chemical properties of the preparation, the dose, the method of administration, the medicinal form, duration of administration (singly or in multiple administrations), the functional status of the organism, etc.

When certain preparations are taken, the pharmacological effect (more exactly, the consequences) may be detected after several hours. Thus, for example, 15 hours after taking sleeping pills there is a decrease in working ability among the crew of spacecraft.

The sulfanilamides reduce visual acuity, and reduce the resistance of the subjects to hypoxia.

Tranquilizers reduce the ability of the pilot to withstand high-altitude factors and accelerations. Antibiotics (streptomycin, dihydrostreptomycin) can produce a severe allergic reaction only 15 to 20 minutes after administration, or even after

several days and even weeks have passed (cutaneous perspiration, rash, edema, pain, vestibular disturbances and temporary loss of hearing).

Quinine leads to a temporary disruption of vision, noises in the ears, dizziness and temporary deafness.

The examples which we have presented indicate that improper use of certain preparations by the cosmonaut or pilot before or during the flight may lead to definite preconditions for flight events. This is why uncontrolled self-medication is not allowed.

Before the flight or during the flight, at the physician's discretion, the use of the following preparations is allowed:

— mezatone in the form of nose drops or by inhalation for rhinitis, as well as to prevent aerosinusitis or barootitis;

— absorbents of the activated charcoal type (carbolene) for high-altitude meteorism. One can also recommend garlic for this purpose. It tones up the motor and secretory functions of the gastrointestinal tract and suppresses the fermentation processes in the gut;

— preparations of ammonium chloride or ammonium phosphate for protecting the blood against reactions with regard to alcolytic changes, which complicate dissociation of oxyhemoglobin in the tissue capillaries under conditions of high-altitude flight. However, these preparations sometimes irritate the mucous membrane of the stomach, so that they must be used after a meal;

— Vitamins: B_2 — 3 mg, A — 3 - 5 mg, PP — 30 mg, C — 150 - 250 mg. The vitamin complex is particularly recommended

for space and high-altitude flights. This complex increases the resistance of the organism to hypoxia and other unfavorable flight factors.

To combat signs of fatigue, to increase the tone of the cardiovascular system and working ability and also to increase the resistance of the organism to dynamic flight factors, it is quite justifiable to use stimulants and general toning substances (phenamine, caffeine, strychnine, securenine, etc.). However, we must keep in mind that in 15 - 20% of the cases phenamine, instead of stimulating the central nervous system and raising working ability, may have the exact opposite effect. In addition, prolonged use of phenamine may lead to exhaustion of the central nervous system and addiction.

Preparations of ginseng and eleuterococcus are also good /52
stimulants; unlike phenamine and pervitin, however, they have a gentler action and lack side effects. They are non-toxic. It must be emphasized that ginseng, dibazole and especially eleuterococcus are typical adaptogens, i.e., they are substances which produce a state of non-specific increased resistivity of the organism to factors that are very different in nature — physical, chemical and biological. They have a protective effect in an orthostatic test, overload on the centrifuge, decrease in atmospheric pressure, electrotrauma and effective high temperatures, and prevent the toxic effect of certain poisons: nitrous oxide, carbon monoxide and sodium fluoride. It is particularly important that these preparations, which increase the working ability of the organism, have no significant subjective stimulating effect and do not cause any kind of unfavorable changes in the function of the internal organs or metabolism [2].

It follows from the above that the contents of the onboard medical cabinets are a very serious and responsible task. In

these cabinets, it is necessary to include only the necessary minimum of preparations and medicinal instruments. Under flight conditions the medicine may be taken only under the strictest supervision of carefully worked out medical instructions.

It is not allowed, for example, to use a medicinal preparation which has a positive effect on the vestibular apparatus and simultaneously reduces resistance to radiation or hypoxia. Or, for example, aspirin and pyramidone, which are widely employed in medical practice, must not be included in the onboard cabinet, since they sensitize the organism to ionizing radiation and frequently lead to allergic reactions.

The radioprotective substances which are included must be only those which do not reduce the resistance of the organism to overloads, hypoxia and other flight factors.

In order to eliminate the development of allergic reactions among the crew members during the flight due to taking a specific medicinal preparation, one must know extremely well the individual tolerance of the cosmonaut and pilot with respect to the most important medicinal preparations (stimulants, vitamins, radioprotectors, etc.).

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Sensitivity of Mice to Radioprotectors from the Aminothiol and Indolylalkylamine Series During the Period of Aftereffects from Transversely Directed Overloads

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ABSTRACT. The authors studied the influence of overloads (in magnitudes of 10 units, 15 minutes; 30 units, 15 minutes; and 40 units, 10 minutes) on the sensitivity of mice to toxic doses of cystamine, AET, tsistafos and 5-MOT. Immediately after centrifuging, the authors observed a slight increase in the resistance of the organism to aminothiols (cystamine, tsistafos). After 30 minutes there was a statistically reliable increase in the sensitivity of the mice to cystamine and AET; after one hour, the reaction of the animals returned to its original level. When 5-MOT was administered over 24 hours following overload, the authors observed increased resistance. The increase in the level of the overload (30 units, 15 minutes; and 40 units, 10 minutes) was accompanied by a significant increase in the resistance of the animals to the cystamine .

Such antiradiation preparations as cystamine, AET, 5-MOT and serotonin, in optimum doses, lead to a decrease in the ability to withstand overloads [5]. At the same time, however, under the influence of preliminary action of overloads, there is a considerable change in the sensitivity of the organism to pharmacological preparations, especially narcotics (chloral hydrate, thiopental-sodium), sympathomimetics, cardiovascular substances

(nitroglycerine, strophanthin-K, convasid, papaverine), stimulants of the nervous system (strychnine). This may show up either as an increase or reversal of the reaction of the organism to the preparations [2 - 4].

Earlier we described data on the influence of overloads on the sensitivity of mice to histamine, especially the phase changes in the reactivity of the organism to this protector [1]. In the following, we have presented data on a further investigation of tolerance to antiradiation compounds, particularly from the series of aminothiols and indolylalkylamines under conditions of overload aftereffects.

The experiments were performed on random-bred white mice weighing 20 - 25 grams, of both sexes. The mice were subjected to the effects of transversely directed (back-chest) overloads on a centrifuge with a 4.25 m arm. The magnitude of the overload was 10 and 30 units for 15 minutes and 40 units for 10 minutes. After 5 - 10, 15, 30, 60 minutes, 4 and 24 hours following rotation, the mice were given intraperitoneally cystamine dichlorohydrate (180 - 350 mg/kg), or S,β-aminoethylisothiuronium bromide hydrobromide (AET) (160 - 220 mg/kg), 5-methoxy tryptamine (5-MOT), (180 - 240 mg/kg) and monosodium salt of β-aminoethylthiophosphoric acid (tsistafos) (800 - 1200 mg/kg). The preparations were dissolved in distilled water and injected at the rate of 0.1 ml per 10 grams of weight of the animal. The average lethal dose (LD_{50}) was determined by sampling analysis.

Table 1 and Figure 1 show the LD_{50} dynamics of the radioprotectors at various times following rotation with an overload of 10 units, 15 minutes. Five minutes following the overload there was a slight increase in the resistance to cystamine

TABLE 1. AVERAGE LETHAL DOSE (LD_{50}) OF ANTIRADIATION COMPOUNDS FOR MICE AT VARIOUS TIME INTERVALS FOLLOWING THE ACTION OF OVERLOADS (10 units, 15 minutes)

Preparation	Time after rotation	Number of animals	LD_{50}, mg/kg	Confidence intervals with p = 0.05 mg/kg	Function of slope of victim curve
Cystamine	—	421	244,3 **	236,6 — 251,9	1,21
	5	54	260,6	242,4 — 280,1	1,23
	30	72	213,8 *	199,8 — 228,8	1,19
	60	63	243,8	228,5 — 259,1	1,20
	240	77	240,0	224,3 — 255,7	1,21
	1 day	29	260,0	237,0 — 286,6	1,23
	—	69	190,5	182,3 — 199,1	1,17
AET	5	56	182,2	171,0 — 201,2	1,24
	30	56	166,0 *	155,1 — 177,8	1,20
	60	56	193,2	184,0 — 202,9	1,13
	240	30	198,6	189,0 — 204,1	1,09
	—	90	197,2	187,3 — 207,1	1,19
5-MOT	5	90	227,0 *	218,2 — 235,8	1,14
	30	90	216,8 *	210,5 — 223,1	1,11
	60	59	221,3 *	212,8 — 230,2	1,11
	240	30	220,0	203,7 — 236,3	1,17
	1 day	30	223,9 *	217,3 — 230,5	1,06
	—	100	968,3	913,5 — 1026,4	1,18
Tsistafos	5	84	1127,0 *	1073,3 — 1183,3	1,18
	30	88	1014,0	947,6 — 1085,0	1,19
	60	89	977,2	913,2 — 1045,6	1,19
	240	89	977,2	913,2 — 1045,6	1,19

* $p < 0.05$.

** Commas represent decimal points.

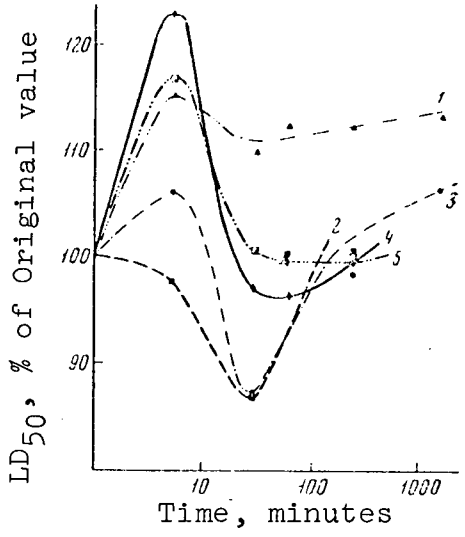

Figure 1. Change in sensitivity of mice to toxic doses of radioprotectors (on the basis of LD_{50}) following action of transversely directed overloads:

1 — 5-MOT (10 units, 15 min); 2 — AET (10 units, 15 min); 3 — cystamine (10 units, 15 min); 4 — cystamine (30 units, 15 min); 5 — tsistafos (10 units, 15 min)

(LD_{50}) = 260.6 mg/kg), while after 30 minutes there was a statistically reliable ($p < 0.01$) increase in the sensitivity of the mice (LD_{50} = 213.8 mg/kg). Sixty minutes later, the reaction of the animals to the administration of cystamine returned to the original level (LD_{50} = 243.8 mg/kg) and remained practically constant for 4 hours after the overloads. However, 24 hours after centrifuging, there was a slight decrease in the sensitivity of the mice to cystamine. The changes in the tolerance of the mice to AET during the early periods following acceleration /55
(10 g for 15 minutes) repeated to a certain degree the dynamics of the change in toxicity of the cystamine under these conditions (see Table 1).

Thirty minutes after centrifuging of the animals, we noticed a statistically reliable increase in the sensitivity of the mice to AET (LD_{50} = 166.0 mg/kg). The restoration of the reaction of the animals to the injection of the protector took place in the 60th minute (LD_{50} = 193.2 mg/kg). During the next three hours, tolerance of AET by the mice did not undergo any significant changes.

The change in the toxicity of 5-MOT for the mice following the action of overloads (10 units, 15 minutes) is shown in Table 1. Five to 10 minutes after rotation, the LD_{50} for 50-MOT increased to 227.0 mg/kg, i.e., it became 15% higher than the original level ($p < 0.05$). The increased resistance of the organism to the radioprotector was retained for 24 hours after centrifuging. Following the action of the overloads, there was a pronounced increase in the resistance of the animals to toxic doses of tsistafos (LD_{50} = 1127 mg/kg). The initial reaction to the preparation returned and did not change for four hours after the action of acceleration (see Table 1).

At an overload equal to 30 units, for 15 minutes, we noticed a statistically reliable decline in the sensitivity of the organism to cystamine (by 23%); later, after 30 - 60 minutes, the sensitivity on the other hand increased and 4 hours later returned to the original level. Thus, when the protector was administered 5 - 10, 30, 60 minutes and 4 hours after rotation, the LD_{50} of cystamine was found to be 300.0, 237.7, 235.5 and 243.2 mg/kg, respectively (Table 2).

An increase in the resistance of the organism to toxic doses of cystamine 10 to 15 minutes following rotation of the mice was also confirmed when exposed to still higher overload values (40 units, 10 minutes) (Table 3).

With exposure to transversely directed overloads, the sensitivity of the animals to antiradiation preparations undergoes a number of phase changes, the nature and direction of whose severity depend on the chemical structure of the compound and the magnitude of the acceleration.

TABLE 2. AVERAGE LETHAL DOSE (LD_{50}) OF CYSTAMINE FOR MICE AFTER VARIOUS TIME INTERVALS FOLLOWING CENTRIFUGING OF THE ANIMALS (30 g, 15 min)

Group	Time after rotation, minutes	Number of animals	LD_{50}, mg/kg	Confidence intervals at p = 0.05, mg/kg	Function of slope of victim curves
Cystamine	–	421	244.3	236.9 - 251.9	1.21
	5	143	300.0*	280.4 - 319.6	1.22
Overload + cystamine	30	120	237.7	233.0 - 242.4	1.06
	60	83	235.5	229.3 - 241.8	1.08
	240	83	243.2	233.8 - 252.9	1.11

*$p < 0.05$.

TABLE 3. TOXICITY OF CYSTAMINE FOR MICE 10 - 15 MINUTES AFTER EFFECT OF ACCELERATION (40 g, 10 minutes)

Group	Dose, mg/kg	Number of animals	Survival, %
Control	270	20	30.0 ± 10.2
Experiment	270	20	85.0 ± 8.0**
Control	320	20	15.0 ± 8.0
Experiment	320	16*	37.5 ± 12.1**

*Four mice died during centrifuging.

**$p < 0.01$.

Thus, for example, immediately after rotation (5 - 10 minutes later) there is an increase in the resistance of the mice to cystamine and tsistafos. It is possible that the increase in the resistance of the organism during this period of time following centrifuging with respect to the radioprotector has to do with the change in the pharmacokinetics of the substance, for example with a slower admission of the preparation into the tissues during disruption of blood circulation in the organs. As the level of the overloads increases, the resistance to the preparation increases as well.

An increase in the sensitivity to antiradiation preparations (cystamine, AET) 30 minutes after rotation apparently is due to exhaustion of the compensatory capacities of the cardiovascular system and respiration due to accumulation of the negative effect of radioprotectors and overloads.

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Influence of Certain Radioprotective Preparations on the Motor-Evacuatory Function of the Gastrointestinal Tract of Healthy and Irradiated Rats

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ABSTRACT. Cystamine, AET, tsistafos and 5-MOT, when administered in optimally protective doses orally, intraperitoneally and into the large intestine, retard the evacuation of contrasting material from the stomach by 4-8, 2, 5-6 and 2-3 times, respectively. Intraperitoneal administration to an animal, prior to irradiation, of cystamine during the first few hours increases the spasms of the pylorus, intensifies disturbance of the evacuatory function of the stomach, and later normalizes the functional state of the gastrointestinal tract.

One of the characteristics of widely known protectors is that /57 their radioprotective action shows up most clearly only with intravenous or intraperitoneal injection. However, their oral administration as a rule leads to a significant decrease in the protective properties [1, 4, 11, 15]. The reasons for this phenomenon have not been completely understood thus far. In this connection, it seems important to us to have a detailed study of the influence of the antiradiation preparations as far as the function of the gastrointestinal tracts of healthy and irradiated animals is concerned. The works which have been published on this topic are

highly contradictory in regard to their results [11, 13, 16, 17, 18, 21, 22]. This once again emphasizes the need to perform some tests in this direction.

The present paper presents some experimental data dealing with a comparative study of the influence of certain effective radioprotective preparations on the motor-evacuatory function of the gastrointestinal tract in healthy and irradiated rats.

The experiments were performed on white rats weighing 170 - 200 grams, which (depending on the nature of the influence to be applied) were divided into 7 groups. In the case of the animals in groups 1 through 4, we studied the influence of cystamine, β-aminoethylisothiuronium (AET), monosodium salt of β-aminoethyl-thiophosphoric acid (tsistafos) and 5-methoxytryptamine (5-MOT) on the function of the gastrointestinal tract. The preparations which we studied were administered orally, intraperitoneally and into the large intestine in optimally protective doses used conventionally during irradiation of this species of animal. In some of the experiments, the dose was reduced by a factor of 2 to 4. The doses of the preparations were calculated for the base. Concentration of solutions of all the substances for each method of administration was the same with the exception of tsistafos: intraperitoneally — 1%, into the stomach — 2%, and into the intestine — 4%. The concentration of the tsistafos solutions was increased by a factor of 2 in view of the fact that they were used at a higher dose per kg of weight.

The rats in group 5 were subjected to total irradiation with γ-rays (Co^{60}) on the "Khizotron" apparatus at a dose of 840 r (11.7 r/min) without chemical protection. The 6^{th} group of animals, 10 - 15 minutes prior to irradiation performed under the same conditions as for the 5^{th} group, received an intraperitoneal injection of cystamine in the optimal protective dose.

Observations of the functional activity of the gastrointestinal tract were performed with the aid of an X-ray method of investigation. The experimental rats received preparations (the controls received a physiological solution) or were subjected to irradiation; then, 15 to 20 minutes later, all of the animals received a contrasting substance (barium sulfate) by a stomach tube at a dilution rate of 1 : 2 in the amount of 5 ml. Then the organs of the abdominal cavity were x-rayed at specific time intervals until the gastrointestinal tract was completely empty. In analyzing the x-rays, we took the following into account: the size and shape of the stomach, its tone, peristalsis, the path of evacuation, the tone of the intestine, the time for complete evacuation of the contrasting substance. The data on the influence of cystamine, AET, tsistafos and mexamine on the motor-evacuatory function of the gastrointestinal tract of the rat are presented in the table.

It is apparent from this table that in all the experimental groups, under the influence of radioprotective preparations, there was a sharp decline in the evacuation of the contrasting substance from the intestine. Complete emptying of the substance upon injection of the preparations orally, intraperitoneally and into the large intestine was observed, respectively, 21 - 40, /59
12 - 31 and 9 - 17 hours later (in the controls, the average time was 4.8 hours). As we can see from the table, the greatest disruptions of evacuatory activity took place in the case of oral administration. When the other methods of administration were employed, especially intraintestinal, the delay was brief.

The x-ray photographs of the experimental rats showed changes in the stomach which were uniform in nature but differed slightly in terms of duration as a function of the method of administration and the nature of the preparation. These changes

TIME FOR EMPTYING (IN HOURS) OF GASTROINTESTINAL TRACT IN RATS UNDER THE INFLUENCE OF RADIOPROTECTIVE PREPARATIONS**

Preparation	Dose, mg/kg	Stomach				Intestine			
		Control	After introduction of preparation			Control	After introduction of preparation		
			In the stomach	Intraperitoneally	In the large intestine		In the stomach	Intraperitoneally	In the large intestine
Cystamine	100 * / 200	5±1,7	40±4,3	31±3,1	17±2,7	27±4,2	62±4,3	53±4,1	20±2,0
Tsistafos	100 / 400	4,8±1,0	38±4,1	25±2,9	10±2,1	21±3,1	68±4,8	44±3,6	20±3,6
AET	100 / 300	4,8±1,1	21±3,1	12±2,2	9±2,0	24±4,2	41±3,0	37±2,8	19±2,0
Mexamine	15 / 50	4,6±1,2	32±3,2	23±1,5	9±1,5	22±3,5	58±3,9	39±2,4	21±2,4

Note: Ten rats per group.

* In numerator — doses with intraperitoneal administration; in the denominator — orally and in the large intestine.

** Commas represent decimal points.

were most clearly evident when the preparations were administered orally. Only 30 minutes later, one could see the presence of spasms in the pylorus and the prepyloric region of the stomach. The longest period of spasms (lasting 8 to 10 hours) was observed

from cystamine and tsistafos. In the case of intraperitoneal administration, the spasms of the pylorus lasted 4 to 8 hours, while the spasms of the prepyloric region of the stomach were also less significant relative to the peroral administration. When the preparations were introduced into the large intestine, there was only a brief pyloric spasm, while spasms of the prepyloric portion of the stomach were usually absent.

The tone of the stomach increased immediately after administration and during the first 1.5 - 2 hours. At the same time, there was an increase in peristalsis. The period of increased motor activity of the stomach was replaced after 1.5 - 2 hours by a prolonged (12 to 18 hour) period of motor activity which in /60
some cases was marked by a still longer attenuation of peristalsis and development of hypotension. Cystamine and tsistafos produced a more significant and prolonged hypotension than did AET and mexamine. Evacuation was completely absent during the first few hours.

In Figure 1, we can see that 3 hours following oral administration of the preparations, the barium in all of the animals had passed into the small intestine only in small amounts, and only a small quantity of contrasting material remained in the stomach of the control rats at this time. After 6 hours, the X-ray picture in the rats which had received cystamine and tsistafos had remained nearly constant. We also noticed the presence of spasms in the prepyloric region of the stomach as well as the absence of peristalsis and evacuation. In those rats which had received AET and mexamine in the presence of spastic phenomena, there was evacuation, although it was slow. However, the stomachs of these animals still contained significant barium residue. In the controls, however, there was complete evacuation.

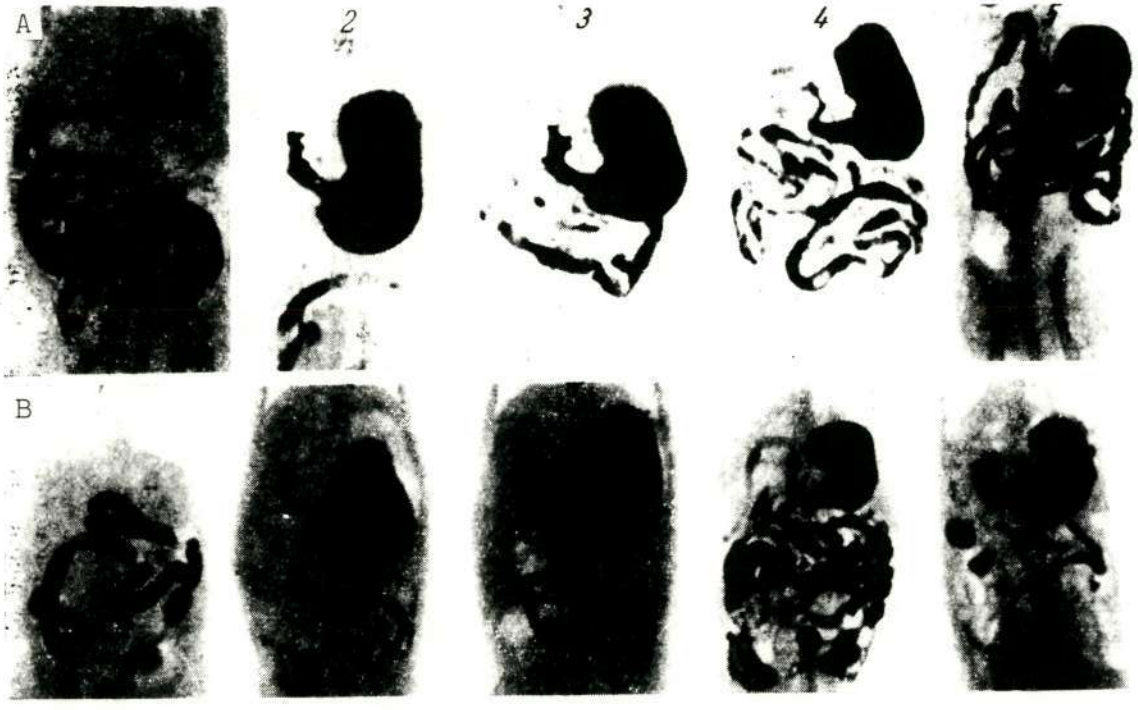

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Figure 1. Tonic and motor-evacuatory functions of the gastrointestinal tract of healthy rats 3 (A) and 6 (B) hours following administration of radioprotective preparations:

1 — control; 2 — cystamine; 3 — tsistafos; 4 — S,β-aminoethylisothiuronium; 5 — 5-MOT

Restoration of the normal motor-evacuatory activity of the stomach in the experimental animals occurred following oral administration usually at the end of the first or second day, and slightly earlier when intraperitoneal administration was used.

It was also important to determine what relationship the observed phenomena of motor-evacuatory function bore to the dose of preparation. For this purpose, we organized two additional series of experiments. In the first series the optimally protective doses were reduced by a factor of 2, and in the second series — by a factor of 4. The preparations were administered orally. An analysis of the results of these experiments showed that regardless of the decrease of the dose by a factor of 2, the cystamine produced essentially the same changes and also retarded evacuation as was the case when optimally protective doses were given. In the case of a two-fold reduction of the dose of residual preparations, evacuation was complete in 12 to 18 hours (as against 21 - 38 in the case of optimally protected animals). If the dose of preparation was reduced by a factor of 4 in the majority of animals, evacuation terminated at the same times as in the controls, and it was only in individual animals, especially with injection of cystamine, that it was retarded by 2 - 3 hours. It is characteristic in this regard that the duodenal spasms were also noticed when such small doses were given, but they were brief (20 - 30 minutes), after which evacuation was completed intensively with increased peristalsis.

According to the data from a number of authors [13, 16, 17], cystamine and cysteamine retard the movement of the contrasting material through the intestine. In our experiments, the introduction of protectors also led to a delay in the evacuation of the intestine. This delay depended essentially on the disruption of the evacuatory function of the stomach and the nonuniform

arrival of barium in the small intestine. However, the movement of the barium suspension directly along the intestinal canals was frequently even accelerated. During the first hours following the introduction of the preparations, we noticed an increase in the tone of the proximal sections of the small intestine. Later, /62 a prolonged dystonia developed.

Hence, the materials examined indicated that radioprotective preparations (cystamine, tsistafos, AET and mexamine) caused disruptions of the motor-evacuatory function of the stomach in rats. These changes took the form of spasms of the pylorus and the prepyloric region of the stomach, as well as a phase disruption of tone and peristalsis: a brief intensification during the initial hours followed by prolonged attenuation. The duration and severity of the changes depend upon the nature of the preparation and the method in which it is administered. These changes probably lead to the prolonged retardation of evacuation. In this connection, we should note that these disruptions of the motor-evacuatory function of the stomach may be of importance in the evaluation of the antiradiation effectiveness of the preparations when they are administered orally.

In the fifth group of rats (total γ-irradiation without chemical protection) we studied the motor-evacuatory function of the gastrointestinal tract for the entire period of observation. Only 30 minutes after irradiation, the animals developed a moderate increase in stomach tone and an intensification of the peristaltic activity. Then, by the end of the first or second hour, a definite spasm had developed in the pylorus, which retarded the evacuation of the stomach. Twenty-four hours later, there was still a small quantity of barium suspension in it. In the small intestine, 2 - 4 hours following irradiation, we could detect signs of dystony, as well as the retardation of the movement of

the contrasting material. Twenty-four hours after irradiation and for 3 - 4 days thereafter we observed pronounced hypotonia of the stomach; its dimensions increased by 3 - 4 times and it contained a large quantity or liquid gas. Evacuation from the stomach was considerably retarded and proceeded unevenly. We could see pronounced dystony in the small intestine. There was an alternation of spastically contracted or hypotonic sections of the small intestine. Along the small intestine, we could see considerable accumulation of gas. The appendix was hypotonic, elongated and contained barium residue for a long time.

Approximately 5 - 6 days following irradiation, the pronounced changes in the animals were replaced by a moderate decrease in stomach tone, intensification of peristaltic activity and a slight acceleration of evacuation. The surviving rats developed gradual normalization that lasted about 2 weeks after irradiation. However, subsequently, up to the 30th day of observation, we could see pronounced lability of the tone of the stomach and intestine, peristaltic and motor-evacuatory functions. In addition to the functional disruptions mentioned above, beginning approximately on the 7th day we began to notice the following changes: unevenness, pronounced serration and scalloping of the outlines of the stomach and small intestine, strong local spasms along the greater curvature and food-like projections along the lesser curvature, as well as the unclear outlines of slight defects in the filling in various parts of the stomach. /63
Autopsy and morphological examination of the digestive organs of animals which died during this period revealed that there were numerous hemorrhages and ulcerations of the mucous membrane along the gastrointestinal tract.

Finally, in the sixth group (intraperitoneal injection of cystamine and subsequent irradiation) we observed characteristic

changes. Only 30 minutes following irradiation, the animals developed spasms of the pylorus and the antral portion of the stomach. This led to a sharp disruption of its evacuatory function. Twenty-four hours later the stomach contained more than 2/3 of the barium administered the day before. Consequently, disruption of the evacuatory function of the stomach during the first few days after introduction of cystamine and irradiation was more pronounced than when these factors acted separately. However, by the end of the second day the function of the gastrointestinal tract had normalized and remained so for 4 - 7 days (Figure 2). During the period of maximum radiation sickness — 7th - 14th days — the functional and organic changes affecting the stomach in the rats of this group were considerably less than with irradiation without administration of cystamine.

These data indicate that γ-irradiation of rats produces pronounced disruptions of the motor-evacuatory and tonic functions of the gastrointestinal tract.

These data are in complete agreement with the reports of a number of authors who found pronounced changes in the digestive system in human beings and experimental

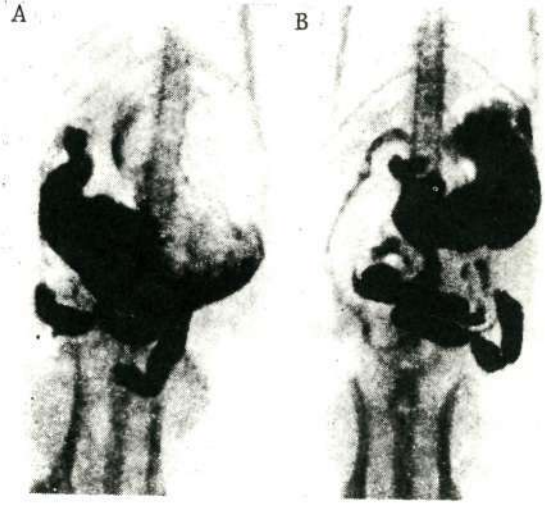

Figure 2. Tonic and motor-evacuatory functions of the gastrointestinal tract of the rat, 3 days after irradiation: A — without protection; B — administration of cystamine prior to irradiation

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animals in radiation damage [2, 3, 5, 10, 14, 19, 20]. Intraperitoneal injection of cystamine in the animals prior to irradiation increases the spasms of the pylorus during the first hours and intensifies the disruption of the evacuatory function of the stomach, and also causes a pronounced protective effect on the functional state of the gastrointestinal tract.

Hence, we have shown that cystamine, AET and mexamine, when introduced in optimally protective doses orally, intraperitoneally and into the small intestine, retard evacuation of the contrasting material from the stomach by factors of 4 - 8, 2.5 - 6 and 2 - 3, respectively.

The protectors which we studied produced prolonged spasms of the pylorus and the prepyloric region of the stomach, as well as phase disruptions of the tone and peristalsis. The severity of these disruptions depends upon the nature of the preparation and the method of administration.

Total irradiation of rats at a dose of 840 r produces sharp changes in the tonic and motor-evacuatory functions of the gastrointestinal tract, which are manifested several hours following irradiation and are observed for a period of 30 days of observation or until the animals die.

Intraperitoneal injection into the animals of cystamine prior to irradiation increases the spasms of the pylorus during the first few hours and intensifies disruption of the evacuatory function of the stomach, and later has a pronounced normalizing effect on the functional state of the gastrointestinal tract.

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Certain Characteristics of the Action of Sympathomimetic Amines on the Reflex Functions of the Central Nervous System of Irradiated and Sympathectomized Animals

P. P. Saksonov

ABSTRACT. Adrenalin, sympathol, veritol, adrenalone, ephedrine, phenamine and Pervitin preferably inhibit the reflex action of the spinal cord. The intensity and the nature of this effect bears a certain relationship to the chemical structure of the amine and the functional state of the central nervous system. The toxicity of all the amines for irradiated animals was increased by a factor of 2 to 4 in comparison to normal. The degree of inhibition of the spinal cord in irradiated animals was approximately the same as in sympathectomized animals.

Sympathomimetic amines as stimulants of the nervous system are of particular interest in connection with space medicine. Therefore, their extensive study is extremely valuable from the standpoint of working out indications and contraindications for their application as preparations which increase the working ability and reduce the debilitating effect of certain medical-prophylactic preparations. It is interesting to study the influence of sympathomimetic amines on the central nervous system

and especially the sympathetic nervous system in various functional states of the organism. Such studies make it possible to use them more rationally and also to penetrate more deeply into the intimate mechanisms of the reactions of the organisms to the action of stressor flight factors.

The sympathomimetic amines of the adrenalin group have an inhibiting effect on the reflex stimulatability of the spinal cord, but this effect is particularly sharp and constant as evidenced by those preparations whose chemical structure lacks hydroxyl groups (phenamine, Pervitin).

Our studies were performed in order to define the character and the intensity of the activity of certain sympathomimetic amines on various parts of the central nervous system; to establish whether or not the change in the reflex function of the central nervous system with sympathomimetic amines has to do with their direct action on it, or with an indirect effect (through the sympathetic nervous system); to explain the significance of the chemical structure of these amines in their physiological action on these reactions, and finally to analyze the modified role of irradiation in these reactions. /66

To solve these problems, we used the following: adrenalin, adrenalone, veritol, ephedrine, sympatol, phenamine and Pervitin.

The selection of these preparations for the purposes of this investigation was based on the following considerations.

All of these preparations possess sympathomimetic properties to one degree or another; one of them (for example, adrenalin) is a true sympathomimetic substance, while the others (phenamine, Pervitin, ephedrine) are pseudosympathomimetic. Therefore, it

was necessary to expect a difference a priori in the force and nature of their effect on reflex functions of the central nervous system. At the present time, it is well known that the substances in the adrenergic group with a small number of hydroxyls, or a complete absence of them in the phenol ring, have far fewer peripheral effects and more central ones.

These preparations have comparatively wide application in practical medicine both as vascular analeptics (adrenalin, ephedrine, sympatol), and also as stimulants of the central nervous system (Pervitin, phenamine).

Their sympathomimetic effect is more or less well-known, and this fact facilitates an evaluation of the results obtained.

It was shown that in large doses all of the amines inhibited the reflex activity. After adrenalin, veritol, to a lesser degree adrenalone, ephedrine, sympatol and in some cases after phenamine, there is a stage of overt excitation which precedes inhibition. In the case of Pervitin and in the majority of experiments with phenamine, the initial period of excitation is absent, and the inhibiting effect begins immediately.

In experiments on spinal frogs, all 7 amines proved to have a predominantly inhibiting effect on the reflex stimulatability of the spinal cord. Amines whose molecule lacks hydroxyl groups (phenamine, Pervitin) had a greater effect on the reflex functions of the spinal cord than did amines with these groups (adrenalin, sympatol). These data are in full agreement with the results of the studies of these amines on other objects by many authors.

On the basis of the degree of the inhibiting effect on the spinal cord of spinal frogs, amines may be divided into the following order in terms of inhibiting power: Pervitin → phenamine → ephedrine → veritol → adrenalone → adrenalin → sympatol. Thus, for example, Pervitin on the average increases the latent period of the reflex by 74.8 and sympatol — by 60.3%. /67

The inhibiting effect of adrenalin on the spinal cord is intensified following preliminary injection of an inactive dose of ephedrine or phenamine. In many cases, with a repeated injection of phenamine, Pervitin, ephedrine, even in increasing doses, and also when administering them following adrenalone, there is a decrease or complete disappearance of the inhibiting effect (tachyphylaxis).

However, in intact animals for all amines (in certain concentrations) the number of reacting animals in comparison to the spinal ones decreases slightly. The number of animals which react by inhibition of reflex activity is less than for the spinal animals. The very nature of the inhibition, as a rule, is not so intensive or prolonged as in the spinal frogs and the response reaction usually takes place earlier. One is struck by the increase in the number of frogs which respond to a stimulus.

All of this unquestionably points to a definite role of the super-segmental portions of the central nervous system in the action of sympathomimetic amines on the reflex functions of the latter. On the basis of the intensity of the inhibiting effect on the reflexes of intact frogs, the amines can be arranged in approximately the same order as for spinal animals.

The "kicking" reflexes are more reliable for sympathomimetic amines than the Turk-Sechenov reflexes. It is only toward the

end of the second hour, when the Turk-Sechenov reflexes are sharply inhibited, that the "kicking" reflex is disrupted.

Large doses of amines not only increase the latent period of the reflex, but also disrupt the ability of the frog to locate the point of irritation precisely. In the majority of thalamic frogs (about 80 - 85%), just as in spinal ones, the clearest and most uniform spinal reflexes occur one and a half to two hours after the operation and remain more or less on the same level for a long time (3 days or more). While in the case of the spinal frog the effect of the amines begins as a rule after 15 to 30 minutes and sometimes longer, in thalamic frogs it takes place in 5 - 10 minutes and as a rule lasts no less than two and a half to three hours. Following injection of the amine, the majority of animals respond not only by an increase in the latent time of the reflex, but also by considerable attenuation of its force.

The generalized reflex process which is usually observed in thalamic frogs following injection of a poison changes to a sharply localized one. As in the case of the spinal animals, there is considerable variability in the sensitivity to a given substance taken at a given concentration.

As far as activity is concerned, the leading place is occupied by Pervitin, followed by phenamine and the third through fifth places are occupied by veritol, adrenalone and ephedrine; the penultimate position is occupied by adrenalin and the very weakest is sympatol. In terms of the average amount of response reactions, the last place is occupied by veritol and sympatol, preceded by adrenalin, while the other four amines have the largest number of response reactions. If we compare these amines in terms of the increase in the latent time of the reflex, we

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again find that Pervitin and phenamine are in first place, while adrenalin and sympatol are last.

The intensity and the nature of the effect of the amines largely depend on the functional state of the central nervous system. The average percentage of response reactions under the influence of all amines in thalamic frogs is greatest. They react to administration of amines primarily by inhibition; this percentage varies from 100 (phenamine) to 81.25 (sympatol), while in the intact animals it is considerably less (from 59.3 to 14%). In the latter, for all amines, inhibition is accompanied by excitation of the central nervous system.

Stimulation in the case of intact animals is also observed when introducing a substance which in thalamic and spinal animals causes only inhibition (phenamine and Pervitin). This is especially evident if we compare the effect of each amine, taken at the same dose, on spinal, thalamic and intact animals. It must be specially emphasized that the sensitivity of thalamic animals in comparison to spinal ones is considerably increased. Thus, the minimum acting concentration of Pervitin for thalamic frogs is $1 \cdot 10^{-7}$, while for spinal animals it is $2 \cdot 10^{-5}$. The same concentration of phenamine for thalamics is 10^{-6}, while for spinal animals it is 10^{-5}.

The "kicking" reflex does not undergo any significant changes, although the Turk-Sechenov reflexes are sharply inhibited. It is only in individual experiments, when the latent period of the Turk-Sechenov reflex is sharply increased (by 200 to 500%), that the "kicking" reflex is either absent or sharply disrupted.

These experimental results clearly indicate the role and significance of the optic thalamus (as well as other parts of the brain) in the action of sympathomimetic amines on the reflex activity of the brain. Removal of the fore-brain (hemispheres) decreases the sensitivity of the spine to the sympathomimetic amines, while thalamic frogs do not react to them by stimulation. In the latter, sensitivity to amines decreases; the spinal cord under these conditions does not respond to the introduction of amines merely by inhibition, but by excitation as well. A. I. Kuznetsov [11, 14] showed that, when the upper cervical sympathetic nodes are removed in the cat, there is a very marked attenuation of both peripheral and central effects of phenamine. His fellow workers showed that protozoa [17], worms [10] and amphibians [4] respond to the influence of sympathomimetic amines /69 primarily by inhibition, while higher animals (especially man) respond by excitation [2, 6 - 8, 11 - 14].

The data which we obtained may provide a better interpretation of the influence of the sympathomimetic amines on the central nervous system. It is highly possible that the sympathomimetic amines act on the central nervous system both directly and indirectly through the sympathetic nervous system. Thus, the toxicity of all amines for the sympathectomized frogs (all rami communicantes on both sides were cut, including the n. hypoglossi) was 1.5 to 3.3 times higher in comparison with normal animals. The effect of sympathomimetic amines on the reflex activity of the spinal cord of sympathectomized frogs decreases to a considerable degree.

The activity of Pervitin and phenamine decreases especially for thalamic frogs, next "intact" animals, and to the least degree for spinal animals. In the case of the three other amines there is a decrease in the activity primarily for the

"intact" animals, next the spinal ones, and for thalamic animals it is much less. However, all of the amines primarily cause changes in the reflex activity of the spinal cord in thalamic frogs in much lesser concentrations than for "intact" animals and especially for spinal frogs.

In no case did we observe any excitation of the spinal reflexes in sympathectomized frogs after introduction of these amines, while in non-sympathectomized animals we observed excitation or initial stimulation with subsequent inhibition in certain cases. This was particularly evident in intact frogs following administration of adrenalin, sympatol and ephedrine, about which we spoke earlier in detail.

Hence, the effect of sympathomimetic amines on the central nervous system bears a certain relationship to the sympathetic nervous system.

We were also interested in the influence of the adrenals on the action of amines, inasmuch as there is a close relationship between the latter and the sympathetic nervous system [1, 5, 9, 15, 18].

There is an indication that the increased function of the cortical layer of the adrenals may to a certain extent lead to a failure of the sympathetic nervous system [5, 21]. As our studies showed, the central nervous system in frogs, which lack adrenals, becomes less reactive to these amines. Sympathectomy in combination with removal of the adrenals leads to a still greater drop in the reaction of the spinal cord to amines.

Yohimbine and ergotamine in doses of 0.5 mg begin to produce inhibition in the reflexes of the spinal cord of thalamic

frogs; at doses of 2 - 5 mg they produce inhibition of the spinal cord in the majority of animals. The degree of inhibition is not always dependent on the dose administered. In no experiment did we observe stimulation of the spinal cord. These paralyzers of the sympathetic nervous system sharply reduce the action of the sympathomimetic amines on the spinal reflexes.

Consequently, ergotamine and yohimbine, like sympathectomization by surgical means, not only increase the effective dose of amines several fold, but decrease the number of reacting animals several fold.

It should also be pointed out that while the group of control animals, following injection of sympathomimetic amine, contains several frogs (about which we have already reported) that show stimulation of the spinal reflexes (especially in intact frogs following injections of adrenalin), this was not observed following administration of ergotamine or yohimbine.

The effect of sympathomimetic amines on the reflex functions of the central nervous system is still further attenuated if the adrenals are removed from the frogs 3 - 4 days prior to the experiment in addition to the administration of ergotamine.

Ergotamine like sympathectomization (even slightly more) intensified the toxic effect of sympathomimetic amines. If an absolutely lethal dose of adrenalin for intact frogs is taken as unity (2.5 mg), we see that for sympathectomized and ergotaminized frogs it will decrease by a factor of 2.5. The absolute lethal dose of phenamine for the former will be decreased by a factor of 3 and by a factor of 3.7 for the latter.

An increase in the toxicity of phenamine and adrenalin for ergotaminized frogs can hardly be explained by a cumulative effect (or even intensification) of the toxic effect of sympathomimetic amine and ergotamine, since in the first place we did not observe the death of frogs from 10 mg of ergotamine. In the second place, phenamine in a dose of 12 mg (i.e., 1.5 times higher than the absolute lethal dose of this substance for ergotaminized frogs) does not lead to the death of intact frogs. The picture of poisoning in ergotaminized frogs following administration of toxic or lethal doses of the preparations mentioned above is the same as for (surgically) sympathectomized frogs, i.e., there is no initial stage of general motor excitation and no production of secretion from the skin glands, while in intact animals adrenalin causes profuse production of secretion.

There are indications in the literature that an increase in the latent period of the reflex which is observed in frogs (using the Turk-Sechenov method) following injection of sympathomimetic amines is due to the fact that these substances have a local anesthetic effect.

It is impossible to treat the changes in the reflex activity of the central nervous system observed following injection of sympathomimetic amines only in terms of their local anesthetic /7
effect, inasmuch as the latter is found only at very large concentrations, and only when applied to the exposed nerve, while inhibition of reflexes following injection of amines is observed at concentrations which are 50 - 100 times less than for local application. In addition, from the standpoint of the local anesthetic effect, it is impossible to explain those cases in which there is excitation instead of inhibition of the reflex activity of the spinal cord. The data of Genes and Bekker [4] contradict this view.

In the experiments performed earlier, one can clearly see the significance of the sympathetic nervous system in the action of these amines on the spinal reflexes of Amphibia. It must be supposed that these amines exert their influence on the central nervous system primarily through the sympathetic nervous system, not excluding the possibility of an indirect effect produced by them on the spinal cord and the subjacent portions of the central nervous system.

To support the first assumption, we can mention the following points:

a) a sharp decrease, as well as a change in the effect of the sympathomimetic amines on the reflex functions of the central nervous system following surgical or pharmacological sympathectomization of the animal;

b) a significant variability in their effect on intact animals. This variability of the results indicates the participation in this effect of the sympathetic nervous system, since in this system, as for the cerebellum, it is characteristic to have an effect that proceeds in two opposite directions, depending on the background against which the influence occurs [15].

As proof of the direct action of the sympathomimetic amines on the central nervous system, we can mention a number of facts such as the following:

a) complete sympathectomization of the animal, although it leads to a sharp decrease in the reactivity of the central nervous system to these amines. Nevertheless, this reactivity is still retained to some extent;

b) both the nature and magnitude of the action of these substances on the spinal reflexes change under the influence of the superjacent portions of the central nervous system;

c) the presence of a stimulating or inhibiting effect (depending on the dose) of the amines and particularly adrenalin on the function of the central nervous system when the substance is applied to the isolated spinal cord of the frog [3].

Our data are in complete agreement with the data of A. I. Kuznetsov [11, 12], who found that the effect of phenamine on the central nervous system of the cat following bilateral extir- /7
pation of the upper cervical sympathetic nodes not only sharply inhibits, but also changes the nature of this effect, in particular eliminating the initial stage of excitation.

Earlier we mentioned that there is a close physiological bond between the sympathetic nervous system and the adrenals. Now we assume that there is a decrease in the stimulatability of the terminal sympathetic apparatus in animals which lack adrenals. In particular, as shown by Kibyakov, there is no production of sympathin under these conditions. However, the function of the adrenal cortex in turn depends on the sympathetic nervous system. Thus, there is a sharp decline in the content of adrenalin in the adrenal veins [1, 7, 15, 21] in animals following cutting of the n. splanchnici.

The toxicity of the sympathomimetic amines for sympathectomized frogs increases sharply, while their effect on the spinal reflexes of these animals, on the contrary, is attenuated to a considerable degree. At first glance, this phenomenon may appear very strange and contradictory, but the contradiction is only apparent. It is completely explicable by the theory of L. A.

Orbeli regarding the adaptational-trophic role of the sympathetic nervous system.

It is worth noting that repeated administration of Pervitin and phenamine to sympathectomized frogs does not lead to the phenomenon of tachyphylaxis.

It was shown by studies of Soviet and foreign authors that the reactivity of irradiated organisms to medicinal substances is supported by considerable changes both in terms of an increase as well as a decrease, and frequently inversion. It is precisely these changes in the reactivity which bear a certain relationship to the degree of severity of the radiation sickness and the period of its development.

In this connection, it seemed to us completely justifiable to perform some special experiments in order to clarify the nature of the effect of the sympathomimetic amines on the reflex functions of the central nervous system of irradiated animals. We assumed that the results of these studies would be of definite interest to space pharmacology.

As we can see from Table 1, the amines which we studied can be arranged in the same order of toxicity as for normal and sympathectomized frogs. As before, first place in terms of toxicity is occupied by adrenalin, and sympatol is last.

The toxicity of all amines for irradiated animals increases by a factor of 2 - 4 in comparison with its toxicity for normal animals, and the irradiated ones were even slightly less resistant to the toxic doses of amines than the sympathectomized ones.

It should be mentioned that irradiated frogs can withstand surgical intervention poorly. In preparing the thalamic and especially the spinal preparations, we suffered a large percentage of deaths (25 - 35% of the thalamic frogs and 40 - 45% of the spinal ones). It was difficult to stop hemorrhaging in the spinal and thalamic frogs. The latent time of the reflex in the irradiated frogs was longer than for the normal ones and even the sympathectomized ones. /7

One and a half to two hours following the start of the experiment, the increase in the latent period of the reflex had a stepwise character, as in sympathectomized frogs.

TABLE 1. COMPARATIVE TOXICITY OF AMINES FOR IRRADIATED (EIGHTH DAY AFTER IRRADIATION AT A DOSE OF 2,000 r), SYMPATHECTOMIZED AND HEALTHY FROGS

Amines	Lethal doses, mg		
	Irradiated	Sympathectomized	Healthy
Sympatol	50	60	130
Ephedrine	15	20	30
Phenamine	8	10	30
Pervitin	5	6	20
Adrenalin	1	1	2.5

For a comparative idea of the effect of the tested substance on the reflex activity of the spinal column of irradiated frogs, as in the previous experiments, we tried to find the minimum dose which could have a significant influence on the excitability

of the spinal column, by no less than 66%. The effect of all seven amines on the reflex activity of the spinal cord of irradiated animals, in comparison with normal ones, was decreased to a considerable extent (Table 2).

TABLE 2. MULTIPLICITY OF INCREASE IN DOSE OF AMINES FOR IRRADIATED FROGS IN COMPARISON TO NONIRRADIATED ONES

Amines	Thalamic	Spinal	Intact
Pervitin	100	20	33
Phenamine	33	10	16
Ephedrine	12	12	10
Veritol	10	10	8
Adrenalone	12	10	12
Adrenalin	5	12	5
Sympatol	5	10	5

It is evident from Table 2 that the activity of amines in the majority of irradiated frogs is apparent to even a lesser degree than in sympathectomized frogs, especially in thalamic and spinal preparations. The degree of inhibition of the spinal cord (with an increase of the latent period of the reflex) under the influence of amines in irradiated animals was approximately the same as in sympathectomized ones.

With repeated administration (2 and 4 times) of Pervitin, phenamine and ephedrine to irradiated thalamic and spinal animals we did not observe tachyphylaxis. Hence, the effect of sympathomimetic amines on the reflex functions of the central nervous

system in irradiated animals was found to be approximately the same as for sympathectomized ones. This apparently is completely in order, inasmuch as ionizing radiation in harmful doses leads to significant disruption of the functions of all the systems of the organism, especially the nervous and endocrine systems, which in the final analysis causes changes in the reactivity of the irradiated organism to various environmental factors, including medicinal substances. Hence, the experimental data presented in this paper indicate that all seven investigated amines have an essentially inhibiting effect on the reflex functions of the central nervous system. The force and the nature of this effect have to do with their different chemical structures and the functional state of the central nervous system. The sympathetic nervous system plays an important role in the action of amines on the reflexes.

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N73-20064

Characteristics of the Action of Medicinal Preparations Under Hypothermic Conditions

B. G. Volynskiy and S. L. Freydman

ABSTRACT. Caffeine, Euphyllin, cordiamin or hypothermy (rectal temperature of +19 or +20° C) leads to an inhibition of respiration, a drop in the arterial blood pressure, disruption of the bioelectrical activity of the heart and metabolic processes in the organism. There is also a drop in the content of ATP and glycogen in the tissues of the heart, liver, kidneys, and brain. There is a tendency toward an increase in excretion of potassium ions from the organism in the urine. Morphine under conditions of hypothermy does not increase the inhibiting action of low temperature on the heart activity and the state of the metabolic processes in the tissues, while potassium chloride causes a sharp drop in the arterial blood pressure. Adrenalin and mesaton retain the nature of their activity in hypothermy.

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In recent years, artificial hypothermy, successfully used for various operations in clinics, has attracted the increasing interest of specialists in space biology and medicine. This interest is related not only to the desire to clarify the action of hypothermy on various functions of the organism, in order to be able to work out the most effective means of protection for emergency situations during spaceflights, but also to the need to completely control hypothermy in order to use it for ultralong spaceflights lasting several years and perhaps even decades.

In this paper, we set ourselves a very narrow goal — to study the characteristics of the action of widely employed medicinal substances used in practical medicine on the functional

indices of respiration and blood circulation and their relationship to the state of the metabolic processes of the organism during hypothermy.

For this purpose, we studied caffeine, Euphyllin, cordiamin Adrenalin, mesaton, morphine and potassium chloride. The studies were performed on intact rabbits at normal body temperature and under conditions of deep hypothermy (rectal temperature = +19 - +20°). The medicinal substances were administered intravenously at a constant rate. We recorded the arterial blood pressure and the respiratory movements; in a special series of experiments, we made a synchronous recording of the cardiac contractions and the changes in the arterial blood pressure. We performed dynamic electrocardiographic tests (the EKG was recorded with the three standard leads).

In order to study the peripheral action of caffeine and Euphyllin on the blood vessels with the body temperature and the temperature of the ambient medium equal, we performed experiments on the completely isolated ear of the rabbit using the method of Kravkov-Pisemskiy, and on an ear isolated humorally with retention of central innervation. The study of the central effect of caffeine and Euphyllin on the lumen of the peripheral vessels under conditions of deep hypothermy was performed using the method of M. P. Nikolayev.

As the criterion for the action of selected medicinal preparations on the metabolic processes in the organism, we used the number of potassium and sodium ions with calculation of the K/Na coefficient in the plasma, erythrocytes and urine with a parallel determination of the electrolytes under study in the organs (brain, heart, liver, kidneys) using the method of flame

photometry [3]. We also studied the content of glycogen and ATP [1], the activity of adenosine triphosphatase and succino-dehydrogenase [2], the content of fat and water (the method of Soxhlet in the Rushnyak modification), ribonucleic and desoxyribonucleic acids [4] in the tissues of the heart, liver, kidneys and brain.

The changes in the EKG under conditions of hypothermy are characterized by a considerable reduction of the number of cardiac contractions, an increase in the amplitude of the R spike, a lengthening of the PQ and QT intervals, and a decline in the systolic index (Figure 1). The plasma shows a tendency toward an increase in content of potassium ions, and there is an increase in sodium ions in the erythrocytes. Excretion of these electrolytes with the urine decreased slightly. There was an increase in the content of potassium ions in the heart tissues and of sodium ions in the kidney tissues. The ATP content in the tissues of the heart, liver and kidneys did not differ significantly from that in the controls; it was only in the brain tissues that the content of ATP showed a decrease. The activity of adenosine triphosphatase and succino-dehydrogenase showed a clear decline in the muscle of the heart, liver, kidneys and brain tissues. The glycogen content decreased in the tissues of the heart and liver with a tendency toward an increase in the tissues of the brain and kidneys. The content of the fat, water, RNA and DNA in all of the investigated organs did not undergo any significant changes.

Under conditions of deep hypothermy, the caffeine (10 and 100 mg/kg) inhibits respiration and produces a significant and stable decrease in arterial blood pressure (Figure 2).

The effect of caffeine depends on the dose, concentration of solution administered and depth of hypothermy; an increase in the

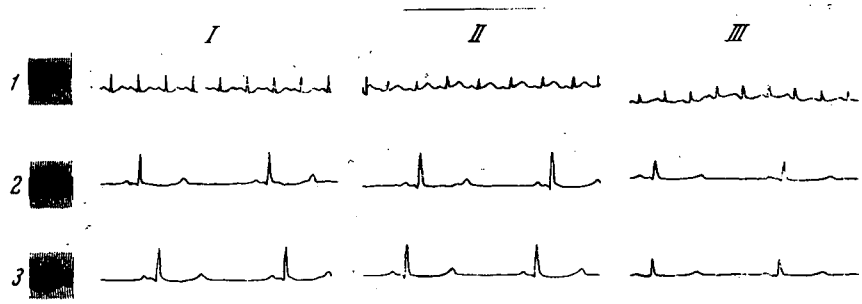

Figure 1. EKG of rabbits in a state of hypothermy (+ 20°):

I, II, III - standard leads; 1 - original state; 2 - beginning of experiment; 3 - 20th minute (end of experiment).

dose and concentration of administered substance reinforces the development of the distorted effect described above.

Regardless of the great inhibiting action of caffeine on the respiration and blood circulation under conditions of deep hypothermy, it evidently does not create changes in the activity of the organism which are incompatible with life, inasmuch as the animals can exist for a long time under artificial respiration.

When independent respiration ceases, arterial pressure does not fall to zero if the life of the animals is maintained by artificial respiration. It is obvious that in deep hypothermy the respiratory center is inhibited more strongly and rapidly than the vasomotorial.

The inhibiting effect of caffeine on blood circulation and respiration under conditions of deep hypothermy is found at those doses at which these functions are evoked in intact animals.

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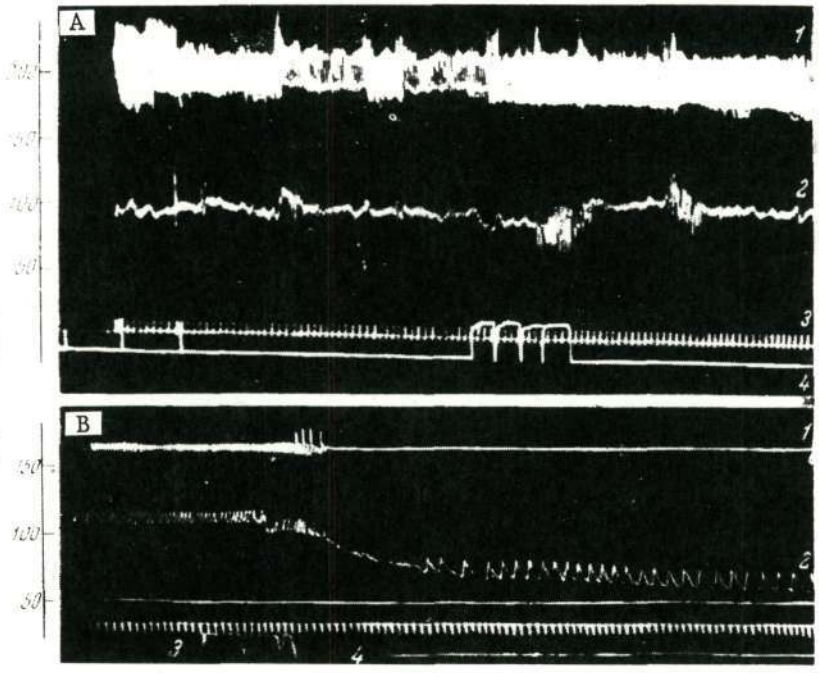

Figure 2. Effect of a 2% solution of caffeine on respiration (1) and arterial pressure (2) in the intact rabbit (A) and in a rabbit in a state of hypothermy (B).

3 - time, 2 seconds; 4 - mark indicating administration of preparation. The caffeine dose for the intact animal was 40 mg/kg and 20 mg/kg for the experimental one.

As far as the effect of caffeineon the lumen of the vessels of the completely isolated rabbit ear and the lumen of the ear vessels (isolated with retention of central innervation under conditions of hypothermy) is concerned, direct application of caffeine on the vessel wall in a dilution of 1:5,000 creates a moderate vasodilatory effect. No significant changes were observed in the action on the blood vessels of a completely isolated ear

or an ear isolated in a vascular respect, but with retention of the central innervation. Consequently, the effects as far as the 78
central nervous system are concerned do not change the sensitivity of the blood vessles to the direct vasodilatory effects of caffeine. Caffeine introduced into the general circulation during deep hypothermy changes the lumen of the blood vessels of the isolated ear. The effect of the preparation under these conditions on the blood vessels takes the form of an expansion or constriction of the vessels, and the vasodilatory effect is predominant. As the experiment continues, the intensity and nature of the effect of caffeine on the blood vessels may change. The intensity of the vasodilatory effect may be very apparent, exceeding the extreme value of the vasodilatory effect under the direct influence of the preparation on the vessel wall.

Under conditions of hypothermy, caffeine produced definite disruptions in the heart activity: an increase in stimulatability and a decrease in the length of the PQ interval, with a tendency toward an increase in the systolic index. It should be mentioned that the use of caffeine during hypothermy is accompanied by a disruption of the rhythm, development of ventricular extrasystole, and in many cases by the development of auriculo-ventricular blockade of the II-III degree (Figure 3).

Under conditions of hypothermy, caffeine significantly influenced the content of electrolytes: the content of potassium ions showed a definite decrease in the plasma, erythrocytes and /79
tissues of all investigated organs with increased excretion of potassium ions in the urine; at the same time, however, the content of sodium ions in these organs increased. It should also be pointed out that there was a definite decrease in the ATP content in the tissues of the heart and liver under the influence of

these doses of caffeine, and in the tissues of the brain and liver with a slight increase in the activity of adenosine triphosphatase (see the table). Under the influence of caffeine at a dose of 10 mg/kg, we observed a decrease in the content of glycogen in the tissues of the heart, brain and kidneys. Under the influence of caffeine at a dose of 100 mg/kg we observed it in the tissues of the liver, kidneys and brain. The succino-dehydrogenase activity of all the investigated organs did increase under the influence of these caffeine doses, but did not reach the level which is characteristic for intact animals. The content of RNA and DNA in the tissues of the investigated organs did not undergo significant changes.

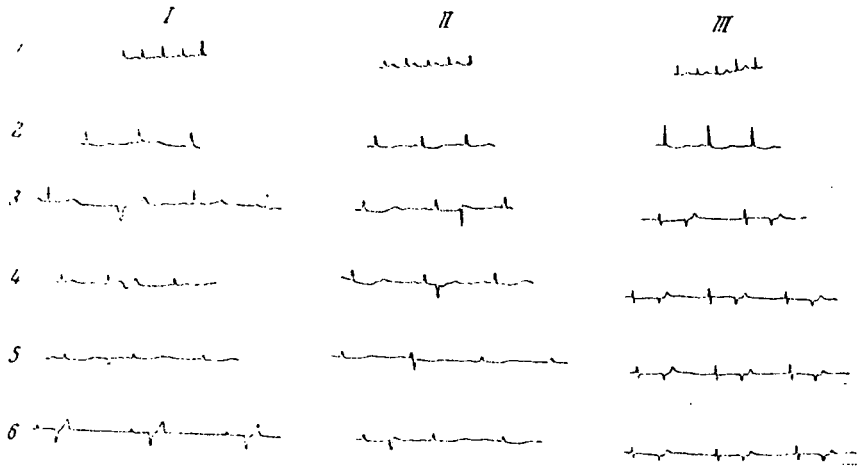

Figure 3. EKG of a rabbit under the influence of caffeine (10 mg/kg) under conditions of hypothermy.

I, II, III - standard leads; 1 - original state; 2 - hypothermy (+20°); 3 - first minute; 4 - fifth minute; 5 - tenth minute; 6 - 15th minute following administration of caffeine.

Hence, caffeine under conditions of hypothermy produces a constant decrease in arterial blood pressure and considerably weakens external respiration. During hypothermy, caffeine definitely increases the load on the heart, produces clear disruptions of the functional capacity of the myocardium and leads to an accumulation of sodium ions, and a decrease in the potassium ion content, ATP and glycogen in the myocardium. Similar biochemical changes were observed in the tissues of the brain and to some extent in the tissues of the liver and kidneys. In many experiments, in which caffeine was employed under conditions of hypothermy, spasms developed, during which there was considerable expenditure of the energy reserves of the organism.

CHANGES IN CERTAIN BIOCHEMICAL INDICES (IN % OF INTACT ANIMAL) IN TISSUES OF THE HEART UNDER THE INFLUENCE OF MEDICINAL PREPARATIONS UNDER CONDITIONS OF HYPOTHERMY

Indices	Hypothermy, 20°	Effect of medicinal substances (in mg/kg) against a background of hypothermy				
		Caffeine, 10	Euphylin, 4.8	Cordiamin, 25	Mesaton, 0.1	Morphine, 1
Amount of ATP	92.8	22.8	24.5*	24.8*	91.5	86.5
Activity of adenosine triphosphatase	28.0	59.0	43.9*	53.0*	42.2*	29.5*
Amount of glycogen	63.5*	55.0*	73.3*	57.9*	136.0*	78.9*
Activity of succinodehydrogenase	36.8*	47.3*	24.6*	59.7*	84.0*	42.5*
Amount of potassium ions	112*	76.9*	77.0*	79.5*	82.0*	113.0
Amount of sodium ions	101	125.0*	131.0*	110.6	71.9*	91.4

*$p < 0.05$

All of the above indicates that it is necessary to exercise /80 extreme care when recommending the use of caffeine under conditions of hypothermy.

Under conditions of deep hypothermy at an Euphyllin dose of 0.48 mg/kg (0.48%, 1 ml/kg), there is a slight increase in arterial pressure, expressed to different degrees in all experiments. At a dose of 2.4 mg/kg (0.24%, 1 ml/kg) there is a hypotensive effect of the preparation which is more strongly evidenced than in intact rabbits. At a dose of 24 mg/kg (2.4% ml/kg) there is a considerable decline in the arterial pressure. At a dose of 2.4 mg/kg, immediately following administration of the preparation, there is a considerable increase in the pulse rate, and at a dose of 24 mg/kg, there is a tendency toward slowing of the pulse. At doses of 0.48 and 2.4 mg/kg, the differences in the effect of Euphyllin on respiration with respect to the controlled experiments are insignificant, but at a dose of 2.4 mg/kg under conditions of hypothermy, we observed brief periods of a marked decrease in the amplitude of respiratory movements. At a dose of 24 mg/kg, in contrast to the controlled experiments, Euphyllin under conditions of deep hypothermy inhibits respiration, decreases the amplitude and frequency of respiratory movements and in individual cases leads to a complete stoppage of respiration. Under conditions of hypothermy, Euphyllin has a direct vasodilatory effect on the blood vessels of the completely isolated ear and the ear with retention of central innervation. We did not find any considerable differences in the peripheral vasodilatory effect on Euphyllin as a function of the presence or absence of a connection with the nerve centers. Under conditions of deep hypothermy, Euphyllin has a central constricting or dilatory effect on the peripheral blood vessels. The nature and intensity of the central vascular effect of Euphyllin may change in a given animal during

various periods or with repeated administration of the preparation. The central vasodilatory effect of Euphyllin may considerably exceed in intensity its peripheral effect.

According to the data from electrocardiography, the effect of Euphyllin is accompanied by pronounced disruptions of the stimulatability and automatism. Under the influence of Euphyllin at a dose of 4.8 mg/kg, there was a rise in the systolic index and a slight decrease in the latter at 24 mg/kg. There was a decrease in the content of potassium ions in the plasma, erythrocytes and tissues of the heart and brain with increased excretion of potassium ions with the urine. There was a tendency toward accumulation of sodium ions in the tissues of the heart, liver, kidneys and brain with a tendency toward a decrease in concentration of sodium ions in the urine.

The content of glycogen in the myocardium showed a tendency toward an increase. In the tissues of the heart, there was a decrease in the content of ATP with an increase in the activity of adenosine triphosphatase. There was a decrease in the content of ATP and glycogen in the tissues of the brain, with a tendency toward an increase in activity of adenosine triphosphatase and succino-dehydrogenase. These changes were accompanied by a general tendency toward a decrease in the content of ATP and a rise in the activity of adenosine triphosphatase and succino-dehydrogenase and an accumulation of glycogen in the tissues of the liver and kidneys with retention of the fat content and nucleic acids in the tissues of all investigated organs at the level of hypothermy. /81

Hence, Euphyllin under conditions of hypothermy has a hypotensive effect and greatly inhibits the external respiration, as

well as causing disruption of electrical activity of the heart, decreasing the content of ATP in the heart muscle and the tissues of the brain, and increasing the excretion of potassium ions from the organism.

Cordiamin at all investigated doses (from 25 - 50 mg/kg in concentrations of 0.25 to 25%) under conditions of deep hypothermy produced a considerable hypotensive effect. Differences with respect to control experiments in the action of the preparation on the frequency of the pulse were insignificant. In deep hypothermy, in all versions of the experiments, there was a decrease in the amplitude of respiratory movements under the influence of the investigated doses of Cordiamin. Under conditions of hypothermy, Cordiamin produced significant disruptions of the indices of electrical activity of the heart, frequently producing disruptions of stimulatability (the development of extrasystole, the development of paroxysmal tachycardia). Under the influence of a small dose of Cordiamin, we observed an increase in the systolic index (Figure 4). Under the influence of the investigated doses of Cordiamin under conditions of hypothermy, there was a decrease in the content of potassium ions in the plasma and erythrocytes and a slight increase in the content of sodium ions in the erythrocytes and tissues of the heart, liver, kidneys and brain, with a tendency toward an increase in the excretion of potassium ions in the urine. During hypothermy, under the influence of Cordiamin, reserves of energy products in the organism were depleted, indicated by a decrease in the reserves of ATP and glycogen in the tissues of the heart, brain, and in the liver and partly in the kidneys as well.

Cordiamin leads to an increase in the activity of succinodehydrogenase in the tissues of the investigated organs and a

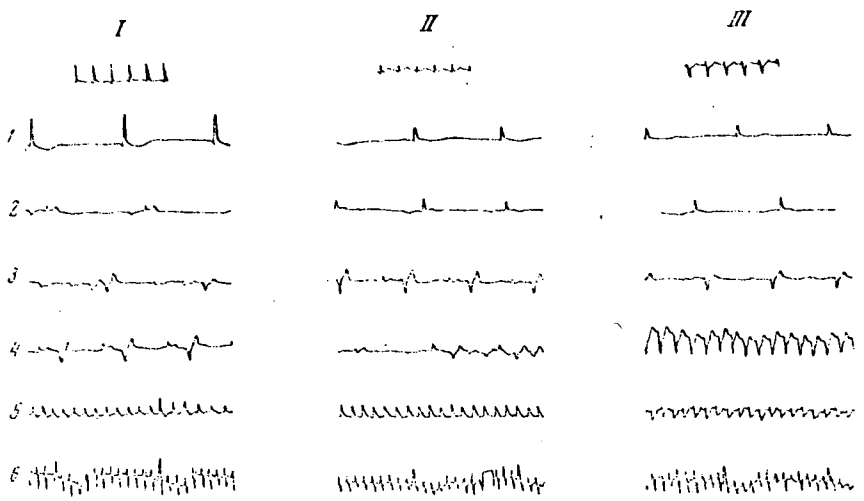

Figure 4. EKG in rabbit under the influence of Cordiamin (25 mg/kg) during conditions of hypothermy.

I, II, III - standard leads; 1 - original state; 2 - hypothermy (+20°); 3 - first minute; 4 - fifth minute; 5 - tenth minute; 6 - 15th minute following administration of Cordiamin.

decrease in the content of fat in the tissues of the kidneys and brain with retention of the content of nucleic acids in the investigated organs. In the spasms which arose under the influence of Cordiamin during hypothermy, the expenditure of the energy resources of the organism can still increase to a considerable degree.

Hence, Cordiamin under conditions of hypothermy considerably lowers the blood pressure, reduces the amplitude of the respiratory movements, causes disruption of the electrical activity of the myocardium, promotes noneconomic consumption of ATP in the tissues and reduces the content of potassium ions in the plasma, erythrocytes, and tissues of the brain, heart, liver and kidneys.

Adrenalin was studied in doses of 0.01 and 0.1 mg/kg. In the state of hypothermy, the principal outlines of the reaction of circulation and respiration to adrenalin are retained (0.01 and 0.1 mg/kg): there is a rise in arterial pressure and a decrease /82 in the amplitude of respiratory movements. At the same time, there are some favorable (from the standpoint of therapeutic value of the preparation) characteristics of the effect: adrenalin during hypothermy exercises a more stable pressor effect, has less of an influence on respiration and pulse rate, and is withstood better by the animals. The intensity of the effect of the adrenalin does not change significantly as a function of the concentration of the administered solution. Hence, a very important characteristic of adrenalin is its ability to maintain its basic action with altered condition of the organism.

Mesaton (0.1 and 1.0 mg/kg) under conditions of hypothermy largely retains its characteristic action on the functional indices of the cardiac activity and metabolic processes of the organism (in comparison with the influence of mesaton in intact animals). According to the electrocardiographic data, mesaton under conditions of hypothermy produced a brief bradycardia, replaced by tachycardia. This change with the use of a small dose of mesaton was accompanied by a tendency toward retention of glycogen and ATP content with a slight decrease in the content of potassium and sodium ions and an increase in the activity of succino-dehydrogenase and adenosine triphosphatase in the tissues of the heart and liver. In the tissues of the heart, liver, kidneys and brain, with use of both doses of mesaton under conditions of hypothermy, the content of RNA and DNA was retained, which may be considered as a favorable factor promoting the recuperative function of the internal systems of the organism upon its emergence from the state of hypothermy. Consequently, mesaton during hypothermy promoted

an economic consumption of energy resources of the tissues, especially the heart. With an increase in the dose of mesaton, its favorable effect may disappear. /83

Hence, the sympathomimetic substances which we studied (adrenalin, small doses of mesaton), exercising a primarily peripheral vasoconstrictory and heart-stimulating effect on the conditions of hypothermy, retain the nature of their action on the functional indices of the heart activity, respiration and metabolic processes in the organism.

According to the data from electrocardiography, morphine in doses of 1 and 25 mg/kg under conditions of hypothermy did not significantly change the frequency of the heart contractions, the magnitude of the PQ and QT intervals, and the systolic index. Hence, the electrical activity of the heart under the influence of both doses of morphine against a background of hypothermy remained at a level which is characteristic for the effect of hypothermy alone. It should be pointed out that in individual experiments involving the study of the effect of a large dose of morphine under conditions of hypothermy we noticed a brief extrasystole. Morphine in both investigated doses did not intensify the inhibiting effect of cold on the activity of succino-dehydrogenase and adenosine triphosphatase, and promoted the retention of ATP, glycogen, and nucleic acid (RNA and DNA) content, as well as fat in the tissues of the investigated organs. The content of electrolytes and the activity of succinic dehydrogenase in the tissues of the majority of investigated organs, especially the heart, was at a level close to hypothermy.

Hence, the effect of morphine on the hypothermy conditions may be rated positively, since morphine did not have an inhibiting influence on the electrical activity of the heart and

promoted the accumulation and economical consumption of macroergs, primarily by such organs as the brain and heart. With a decrease in the body temperature of the animals, we observed an increase in the sensitivity of rats to the toxic effect of potassium chloride, as indicated by the death of the animals which was observed in many cases during hypothermy from doses of 20 mg/kg. This same dose was tolerated well by intact animals.

Under conditions of deep hypothermy, potassium chloride at a dose of 2 mg/kg produces insignificant changes in blood pressure, but after a dose of 20 mg/kg (immediately after administration) there is a fall of the arterial pressure almost to zero. Then the blood pressure rises but does not attain the original level. The fall of blood pressure which is observed with a high dose of the preparation (20 mg/kg) is accompanied by a slowing of the pulse. At a dose of 2 mg/kg, we observed insignificant changes /84 in the frequency and amplitude of respiratory movements, and at a dose of 20 mg/kg there was a slight decrease in the amplitude and frequency of the respiratory movements (Figure 5).

Hence, in hypothermy there is an increase in the sensitivity of the animals to the effect of potassium chloride on blood circulation and respiration. Under conditions of deep hypothermy, we observed a restoration of the level of arterial pressure following significant changes, inasmuch as in this state the organism can better withstand temporary disruptions of the function of respiration and circulation. The fall of arterial pressure takes place earlier than the change in respiration.

On the basis of the studies which we performed, we should point out that under conditions of hypothermy the effect of the investigated medicinal preparations differs markedly from the

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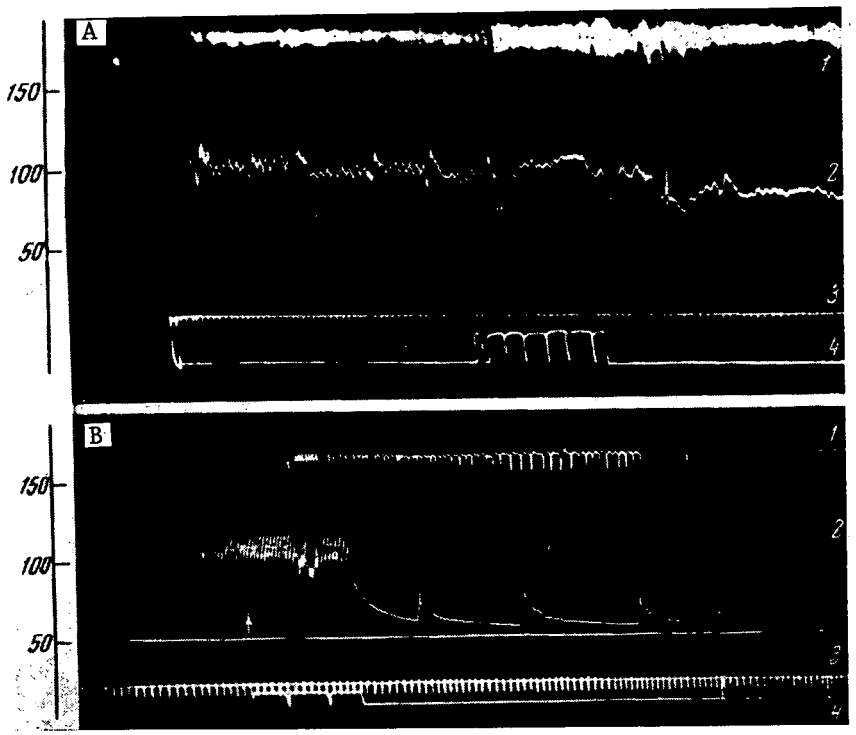

Figure 5. Effect of a 2% solution of potassium chloride in an intact rabbit and in a rabbit in a state of hypothermy (body temperature — 19.5°C). Dose of preparation for intact animal — 38 mg/kg; for experimental, 20 mg/kg.

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generally accepted concepts regarding their influence on the organism. In choosing medicinal substances to be included in the /85 medicine chest, for restoring the functions of respiration and blood circulation, it is necessary to take into account the fact that such substances as caffeine, Euphyllin and Cordiamin may themselves lead to disruptions of cardiac activity and respiration under conditions of supercooling.

On the other hand, under conditions of hypothermy, morphine does not lead to any inhibiting influence on the heart activity. Adrenalin and mesaton are the most reliable substances which have a tonic effect on the functional indices of the circulatory system as well as the biochemical processes in the organism during hypothermy.

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N73-20065

III

INFLUENCE OF RADIOPROTECTORS AND SHIELDING OF VARIOUS PARTS OF THE BODY ON THE REACTIVITY OF THE ORGANISM WITH RESPECT TO IONIZING RADIATION

Chemical Means of Prophylaxis of Radiation Sickness

V. S. Shashkov, B. V. Anisimov and P. P. Saksonov

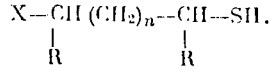
ABSTRACT. In this survey, the authors discuss the principal results obtained in recent years in the use of chemical substances for prophylaxis of radiation sickness both in experiments and in the clinic. The possible mechanisms of action of radioprotectors are discussed.

One of the most important radiobiological problems is the /86
problem of chemical (pharmacological) protection of the human organism against the action of ionizing radiation [6, 17]. At the present time, we know approximately 500 chemical compounds which have an admittedly slight protective effect against radiation. Unfortunately, it has not yet been possible to carry out a scientifically based classification of radioprotectors, although many authors have attempted to organize a classification of this sort [15, 17, 25].

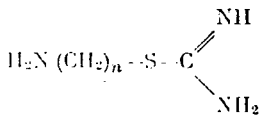
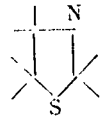
Principal Groups of Radioprotective Substances

Mercaptoalkylamines and derivatives of aminoalkylisothiourea. This group of protectors includes compounds which contain the

sulfhydryl group and a functional grouping of the following basic type:



In addition, it includes mercaptoalkylisothiuronic derivatives:

 and thiazolidines 

Investigations of the dependence of the radioprotective activity on the chemical structure in the mercaptoalkylamine series in recent years have not made any new contributions to the previously discovered rule concerning the maximum activity of β- and γ-mercaptoalkylamines with n = 0 or 1. /87

The study of a great many different derivatives of mercaptoalkylamines, mercaptoalkylguanidines and mercaptoalkylamidines has shown that those which are most effective are the mercaptans which contain the base group in the β- and/or γ-position with respect to sulfur [15, 19, 32]. Lengthening of the carbon chain to more than three atoms of carbon and its branching also reduces the protective effect of the substances, but it still remains high. Complete disappearance of the protective effect is observed with simultaneous N- and S-substitutions.

As we know, the most effective isothiuronium derivatives are AET and APT (aminopropylisothiuronium) in the form of hydrobromides. Monosubstitutes of these compounds in NH_2 differ slightly in their protective effect from the basic preparations, while the dialkyl-substituted derivatives have only a slight protective effect. As a rule, S-alkyl-(aryl)-substituted thioureas have proved to be slightly effective protective substances.

In the group of isothiuronium compounds and mercaptoalkylguanidines, we observed essentially the same characteristics as in the group of mercaptoamines. Similar characteristics are retained in the labile S-ester derivatives of cysteamine: β-aminoethylthiosulfuric acid, propylthiosulfuric acid, β-dimethylaminothiosulfuric acid and β-aminoethylphosphoric acid [1].

A. S. Mozzhukhin et al. [15] concluded that in these groups of substances it would be impossible to find substances that are more effective than MEA, cystamine and AET, although they do not deny the possibility of finding substances which are more convenient for practical utilization than the well-known preparations.

Data on the radioprotective activity of cystamine are very numerous. In experiments on rats, it has been shown that the maximum activity of the substance is observed when it is administered 45 minutes prior to irradiation. With a decrease in this time to two minutes, the protective effect decreases sharply, and the same is true when it is given one hour earlier.

New data have been published on the use of cystamine to protect against early damage to the bone tissue of mice subjected to X-radiation [15]. The protective effect of salts of S, β-aminoethylisothiuronium has been supported in experiments on dogs [30] and monkeys [18]. In mice, irradiated at the minimum lethal doses, AET has a protective effect in mice up to 80 to 90 and even 100% [23, 25], and this effect is retained from 1 to 6 hours prior to irradiation.

Indolylalkylamines. Systematic studies of a number of indolylalkylamines have established a clear relationship between the chemical structure and the radioprotective activity of the

substances. These data were obtained mainly in our country by P. G. Zherebchenko and his associates, by V. D. Rogozkin, and abroad by Langendorf et al. and Dukor et al. /88

The structural base of indolylalkylamines is tryptamine, whose protective action is increased by the addition of substituents in the fifth position. Of the investigated indolylalkylamines, the most effective as far as radioprotection is concerned is 5-methoxytryptamine (mexamine) whose protective effect appears over a wide range of doses (5 - 150 mg/kg) in mice with various means of administration and is retained when administered 5 - 40 minutes prior to exposure. The protective effect of mexamine has been established on rats, dogs and monkeys. The protective effect of 5-methyl and 5-chlorotryptamine is slightly less than for mexamine and corresponds to that of serotonin. An important advantage of mexamine is the retention by the substance of its radioprotective effect when administered orally (250 mg/kg), while serotonin is not effective when so administered. Detailed information on the radioprotective effect of indolylalkylamines and the relationship of their activity to their structure may be found in the doctoral dissertation of P. G. Zherebchenko [23].

Other compounds. Beginning in 1949, numerous attempts were undertaken to find radioprotective preparations among the various classes of known pharmacological preparations, metabolites, their derivatives and analogs, as well as among physiologically active compounds which manifest various biological effects.

In experiments on mice, rats and dogs, the antiradiation properties of substances were studied, primarily those acting on the nervous system, sex hormones, purine and pyrimidine bases, antimitotic substances, alkaloids and antibacterial preparations [16, 18, 22].

There is considerable interest in the work devoted to the study of a new class of radioprotectors: amidophosphothioate and its derivatives. The dilithium salt of S-(3-amino-2-oxypropyl)-phosphothioate and S-(2-aminopropyl)-phosphothioate have been found to be more effective than MEA. In studying substances with the general formula $R_2N(CH_2)_nSPO_3$-, it was found that the protective effect is reinforced when n is reduced. Compounds with n = 0 were synthesized: diammonium amidophosphothioate and diammonium diamidodiphosphate are also more effective in comparison to MEA. The dose reduction factor (DRF) for these substances was 2.3 and 2.16, respectively; the DRF for MEA under these conditions is 1.84. Radioprotectors of this new class produce their protective action in molar concentrations calculated on the basis of the body weight, which are 10 - 20 times smaller than for MEA, i.e., at approximately the same concentrations at which the protective effect of indolylalkylamines is manifested.

Dimethylsulfoxide possesses the unique ability to exert a /89
protective effect when applied to the surface of the body and inhaled in vapor form.

It was found in the work of G. M. Ayrapetyan et al. [1] that N-dithiocarboxycystamine and S-(β-phenylethylcarbomoylethane) thiosulfuric acid exert a protective effect when administered four hours prior to exposure, and a very mild protective effect is maintained even when administered 24 hours prior to exposure.

During the last five years, there has been a considerable increase in interest in studying the protective effect of high-molecular substances: polysaccharides, nucleic acids and proteins. The use of biopolymers for prophylaxis of radiation damage is obviously promising, inasmuch as they are slightly toxic with the

exception of the endotoxins, and effective when administered many hours and even several days prior to exposure. Certain biopolymers exert not only a prophylactic, but also a curative effect. However, the protective effect which is produced by biopolymers is low and mainly unstable. The latter fact obviously is explained by the difficulty of separating and purifying biopolymers. Better results were obtained when using dextrin (an increase in survival rate by 58%), chondroitin sulfate (by 40 - 50%), cyan (by 40%), polysaccharides of various origin (by 10 - 40%), hyaluronic acid (by 27%), heparin (by 13%), nucleic acids (up to 20 - 80% [16, 39]).

Many papers published in recent years have been devoted to a study of the therapeutic effect of nucleic acids [42]. The outlook for practical application of endotoxins as radioprotectors with prolonged effects and therapeutic substances at the present time is doubtful, although experiments on mice have produced satisfactory results [31, 42]. The study of the radioprotective effect of substances acting on the nervous system has revealed the protective effect of imipramine [40].

In recent years, there has been a tendency (mentioned by Thompson [19]) toward a decrease in the number of papers devoted to testing of new radioprotectors on mammals and an increase in the number of works devoted to a study of the mechanism of the action of already well-known radioprotectors.

Chemical Protection Against Corpuscular Radiation

The possibility of chemical protection against radiation from high-energy protons was first demonstrated by S. P. Yarmonenko et al. [26, 27]. The effectiveness of the radioprotectors with respect to proton x- and γ-radiation was approximately the same [41] (see the table).

COMPARATIVE EFFECTIVENESS OF RADIOPROTECTORS WITH DIFFERENT FORMS OF IONIZING RADIATION [21]

/90

Preparation	Survival with irradiation, %		
	γ-rays, 850 r	Protons, 660 MeV, 1170 rad	Protons, 120 MeV, 1170 rad
Cystamine	55	51	60
AET	75	81	75
Serotonin	60	80	35
Mexamine	70	70	70
Tryptamine	20	25	-
5-Oxytryptophan	20	20	-
Control	0	1.7	3.3

According to the data of Vogel et al. [41], serotonin exerted a very slight protective effect in irradiation with fast neutrons. A good protective effect was obtained when using a mixture of MEA and mexamine. With joint administration of these radioprotectors to mice prior to irradiation with protons with energies of 660 MeV at a dose of 1900 rad, 50% of the animals survived, while with separate administration no more than 15% remained [25].

Radioprotective Effect with Combined Use of Protectors

The use of combinations of several radioprotectors and radioprotectors with physiologically active compounds for prophylaxis of radiation damage is governed by the following principles.

First of all, attempts to find more effective radioprotectors have been unsuccessful. Secondly, the existence of different mechanisms has been assumed for the protective action of aminothiols and indolylalkylamines. A combination of protective properties, in the opinion of many investigators, takes place only when each of the investigated radioprotectors has a specific mechanism of action [25]. In the third place, it is governed by the assumption that, although all radioprotectors have a common basic mechanism of action, there are many factors which condition the advisability of joint utilization of several radioprotectors: distribution among the tissues, differences in rate of excretion, etc. [19]. In the fourth place, there is a desire to reduce the toxicity of the radioprotectors.

Attempts to achieve reduction of radioprotector toxicity during their joint application with other pharmacological agents, surprisingly enough, are few. P. P. Saksonov [17] has shown that the toxicity of MEA decreases with simultaneous introduction of nicotinic and ascorbic acids, sodium bromide, aminazine, phenatine, spermine and pyridoxine. Aminazine, according to the data of P. P. Saksonov, not only relieves spasms caused by toxic doses of /91
MEA, cystamine and AET in dogs and cats, but also increases the protective effect of aminothiol protectors by 35%.

P. G. Zherebchenko et al. [12, 13, 14] found that antispasmic substances (Medinal, Luminal, benzonal, Librium) as well as hydrocortisone, reduce the toxicity of the aminothiols. The toxicity of mexamine may be reduced by means of an antihypoxic substance — guanylthiourea. These pharmacological preparations had no influence on the protective effect of radioprotectors.

The administration of thiosulfate and the anion of hypophosphoric acid slightly decreases the toxicity and considerably

increases the radioprotective effect of AET and serotonin. The administration of these radioprotectors with chlorides of Ni, Zn and Co has led to an increase in the toxicity and a sharp decrease in the radioprotective effect of the protectors. The administration of radioprotectors in "carriers" makes it possible to reduce the penetration of the radioprotectors through the hematoencephalic barrier and thereby reduces their toxicity. At the same time, the administration of radioprotectors "on carriers" makes it possible (according to the data of the authors) to increase selectively the concentration of protectors in the marrow and spleen.

When cystamine was administered to mice, adsorbed on cloth or on particles of activated charcoal, the authors observed a decrease in the toxicity of the preparation by a factor of 4 with a slight increase of the protective effect.

One of us (Saksonov, 1953 - 1966) was apparently the first in the USSR to confirm experimentally the prospects for the combined use of radioprotectors.

Later on, studies of the radioprotective activity with combined application of preparations were performed by P. G. Zherebchenko et al., T. K. Dzharakyan, A. S. Mozzhukhin, P. D. Gorizontov, V. D. Rogozkin, Ye. F. Romantsev, V. G. Yakovlev, L. F. Semenov and other authors. In the field of radioprotection, it is important to look for those combinations of radioprotective and pharmacologically active substances which would reduce the toxicity of the radioprotective substance, without changing or increasing the antiradiation effect.

The search for ways of increasing the radioprotective effect by means of the combined use of preparations has moved in different

directions. Cysteamine has been combined with reserpine [32] and triatamine [14], Novocaine with EDTA, (ethylene diamine tetra-acetate) paraaminobenzoic acid and its isomers, aminazine with phenatine, methylene blue with aminothiazol, thiamine with narcotics, cysteine with natural nucleotides and mucopolysaccharides, and certain synthetic polymers. But the tested combinations of these substances were lower in effectiveness than the known radioprotective preparations used separately.

The combination of cystamine with local hypoxia (tourniquet on the extremity) also considerably improved the outcome of radiation at absolutely lethal and superlethal doses [11, 12]. /92
The possibility of increasing the protective effect of aminothiols and AET by means of hypoxia produced by compounds producing metahemoglobin [2, 16] has been shown.

An increase in the radioprotective activity was observed in the combined administration to mice and dogs of indolylalkylamines, tsistafos or AET [1, 10, 14]. There was an increase in the protective effect of l-cysteine by histamine, aminoacetonitrile, tryptamine, 2, 5-dioxyphenylalanine, pyridoxine, glutaminic acid, ATP and sodium cyanide [32].

Considerable interest and great practical significance are attached to works in which a successful attempt has been made to increase the protective effect of aminothiols and other preparations by means of stimulants of the central nervous system and tranquilizers [4], pyridoxine [15], ATP [101, 102] and reserpine [32].

When using mexamine in conjunction with various pharmacological substances, it was shown that aminothiols (MEA, AET) and

narcotics (hexenal) increase their radioprotective properties, while substances which exert a selective influence on the processes of the andrenergic, cholinergic and serotoninergic stimuli reduce the radioprotective effect of mexamine.

There has been great interest in work in which attempts were made to use multiple component mixtures. Basically, these mixtures included combinations of mercaptans with compounds producing meta-hemoglobin, inhibitors of enzymes, stimulants of the central nervous system, and vitamins. Unfortunately, there are only a few such works. They are still more time consuming than testing one or two substances, and their protective effect requires a more complex analysis which involves a considerable expenditure of time and resources.

In experiments on dogs, the high protective effect of a mixture consisting of paraaminopropiophenone, parahydroxyphenyl, mercamine and cysteine [32] has been demonstrated. S. P. Yarmenko concluded that the combined use of mercapto- and indolylalkyl-amines in optimum and reduced doses leads to a considerable increase in the protective effect. The effectiveness of complexes of protectors is found to be equally pronounced over a considerable range of sub-, average and super-lethal doses both with single and fractioned irradiation [25].

A mixture of radioprotectors (MEA, AET and serotonin) reliably protected mice with exposure to repeated irradiation in lethal doses with intervals of 28 days. The DRF values were 2.17, 2.18, 1.95, 2.14 and 1.58, and were obtained for the first five exposures. Protection was limited by the toxicity of the mixture, which turned out to increase with increasing cumulative dose of radiation [42]. /93

It was found in the work of Maisin et al. [36, 37, 38] that a mixture of radioprotectors (glutathione, AET, cysteine, serotonin) exerts a significant radioprotective effect. This combination of protectors provides protection both of the intestine, as well as the lymphopoietic and hematopoietic systems in mice. The authors point out that the use of a mixture of protectors not only increases the degree of protection (on the basis of survival by the 30^{th} day), but also decreases the toxicity of such compounds as AET and MEA.

An analysis of the works in which the radioprotective effect was studied with the combined application of a combination of preparations from different classes of chemical compounds may be continued. However, the results of the studies have been sufficiently completely generalized in a number of monographs and collections [3, 15, 20, 24, 26]. An increase in the radioprotective effect with combined utilization of two or more preparations on all types of animals (mice, rats, dogs, monkeys) indicates the possibilities of this research on protection of mammals against ionizing radiation.

Of particular interest in this connection, and with potential practical significance in our opinion, is the further development of the problem of prophylaxis of radiation damage by means of slightly toxic multicomponent mixtures of protectors and other pharmacological substances.

Possible Mechanisms of Chemical Protection /9

On the basis of concepts regarding the direct and indirect action of radiation, we can isolate the principal aspects of the radioprotective effect of antiradiation substances. These include

protection and inactivation of radicals, protection and oxygen effect, protection by means of physical-chemical changes in the molecules of the biosubstrate, protection by influencing the metabolic processes and influencing processes of recovery. It should be pointed out that many of the previously existing concepts regarding the mechanisms of certain radioprotective substances are contradicted by new facts, and are invalid at the present time. However, even the existing concepts are not always completely satisfactory as far as the basic positions of modern radiobiology are concerned.

Changes in radiosensitivity, which can be produced successfully by means of existing radioprotectors, are many times less than the natural differences for various types of cells and multicellular organisms [8]. The existing level of knowledge cannot provide sufficient accurate criteria for evaluating the limits of /94 changes in the natural radiosensitivity of the organism in mammals by means of chemical prophylaxis of radiation damage.

Protection and Inactivation of Radicals

Beginning with the work of Alexander, Bacq et al. [115] established for more than 100 compounds of various classes the correlation between the degree of their radioprotective activity in vitro (on a polymethacrylate model) and in vivo. A viewpoint which has gained wide acceptance is that concerning the existence of the most general action of protectors, consisting in a concurrent (relative to the protected biosubstrate) capture and inactivation of three radicals, arising under the influence of ionizing radiation. These views are in good agreement with concepts regarding the significant role of the indirect effect of ionizing radiation and are even considered to support this theory.

At the present time, even the founder of this viewpoint, P. Alexander [28] suggests that it is necessary to reexamine the role of inactivation of radicals in the mechanism of protection. He feels that, besides capture of radicals, there are two other possible methods of capture: restoration of damaged molecules by a protector present in the medium, or an increase in the radioresistance of the molecular targets as a result of their temporary bond with the protector.

Even more important arguments against the theory of protection in vivo by means of simple inactivation of radicals may be mentioned. From the standpoint of capture of radicals, it is impossible to understand the wide range of doses of different protectors at which the optimum protection is observed. We have already mentioned that insignificant changes in the structure of the compound lead to a loss of its protective properties. This clearly expressed specificity of mercapto compounds in vivo, mentioned by a number of investigators [34], in comparison to the previous data regarding the approximately uniform ability of these substances to inactivate radicals of water, contradicts the significance of this protection mechanism in vivo. Tryptophane, histidine and thyrozine are just as effective inactivators of radicals as tryptamine and histamine and thyramine, but unlike the latter they do not provide a protective effect in vivo [28].

The protective effect of indole derivatives in vitro does not correlate with their antiradiation activity in vivo, which is therefore hardly produced by this mechanism. Even in such a simple system as a suspension of cells of the thymus gland, some substances such as tryptamine, serotonin, histamine, adrenalin and beta-phenylethylamine do not have an antiradiation effect. Others, such as cysteine, cysteamine, cystamine and ethylenediaminetetraacetate protect the thymocytes against the action of radiation [10].

Protection by Means of Physical-Chemical Changes in the Molecules of the Biosubstrate

This type of mechanism in living cells may be the result of physical and chemical changes in the medium (pH, oxidation-reduction potential, ionic force, temperature) and a chemical combination of molecules of the substrate with organic compounds. These mechanisms have been investigated in detail in the above-mentioned survey of L. Eld'yarn and A. Pil [24], which deals very extensively with the theory of protection by means of formation of mixed disulfides with proteins, proposed by the authors as early as 1957.

It should be pointed out that studies conducted in recent years not only have failed to provide proof supporting this mechanism, but in fact have acted to the contrary, being enriched by data of the opposite kind. Alexander rejects this hypothesis as having no basis and without any direct proof [28]. The disulfide bond may be formed not only by mercaptoethyl- and propylamines but also by their higher homologs, but as we have already pointed out, the latter do not have protective activity.

We know that the mercaptoalkylamines reduce the inhibition of synthesis of DNA which is produced by the action of radiation. These data are supported by findings concerning the analogous influence of AET and cysteine with regard to the marrow in rats and guinea pigs, as well as by results of cytophotospectrometric analysis of small lymphocytes of the spleen of rats protected by cystamine. The data of many authors have also shown that, under the influence of cysteamine, there is a decrease in the early radiation degeneration of the cell nuclei of the marrow and of cells with chromosomal damage. Recently, similar results have

been obtained with respect to AET under the action of protons [26]. All of these data indicate direct protection by means of sulfhydryl compounds of DNA which do not contain mercapto groups. In fact, the materials from these studies also contradict the concepts regarding the concurrent mechanism of protection — i.e., according to the data of Aleksander [28], the indirect effect of radiation with respect to DNA takes place with a concentration of the latter of less than 2% in a solution, while it makes up about 10% in the nucleus [18].

Protection and the Oxygen Effect

The universality of the oxygen effect in various biological systems from cells to man [24] — consisting of the dependence of the damaging effect of ionizing radiation on the oxygen pressure — makes it understandable why there has been an effort by many investigators to link in one way or another the mechanism of the majority of protective substances with hypoxia.

In many works [7, 35] it has been shown that biogenic amines /9 (tryptamine, serotonin, adrenalin, noradrenalin, histamine, beta-phenylethylamine, 5-methoxytryptamine, morphine, heroin, carbon monoxide, sodium nitrite, unithiol, dimercaptopropionic acid) produce a definite change in the oxygen pressure in the tissues, especially in the spleen, determined by means of the polarographic method, on the basis of the time of the maximum protective effect.

For the above amines, this correlates with their vasoconstrictive effect [10, 11, 35]. Other mechanisms form the basis of hypoxia caused by the action of other substances (formation of metahemoglobin — sodium nitrite paraaminopropiophenone; formation of carboxyhemoglobin — carbon monoxide; inhibition of respiratory

enzymes — cyanides; inhibition of respiratory center — morphine, heroin, etc.).

In contradiction to the relationship of the antiradiation effect of a number of these compounds with oxygen effect, there are data on the possibility of attenuation or negation of protection by antagonists, antimetabolites or an increase in oxygen pressure [10, 33].

According to the data of E. Ya. Grayevskiy [7, 8], the basis of the antiradiation effect of hypothermy in homiothermal animals is also the hypoxic mechanism in which the protective effect begins to appear only after a decrease in the oxygen pressure in the tissues below 50% of the original level.

Recently, it has been established that the protective effect of dimethylsulfoxide, chloropromazine and chlorodiazepoxide [32] has to do not with hypothermy but with hypoxia, inasmuch as a decrease in the body temperature produced by these compounds lasts for two hours or more, while the protective effect appears only during the first 30 minutes following administration, i.e., during the time when the decrease in oxygen consumption is observed.

Many individuals suggest that the mechanism of the antiradiation effect of the majority of known protectors evidently has to do in one way or another with the oxygen effect.

At the same time, however, with regard to a large group of thiol compounds (cystamine, cysteamine and AET) there are contradictory data whose detailed analysis is contained in survey articles by E. Ya. Grayevskiy [7, 8].

Many of the studies of Grayevskiy and his associates, summarized in these surveys, lead them to conclude that the protective action of cysteine, cysteamine and AET are independent of the oxygen effect. At the same time, however, dithiols (dimercaptopropionic acid and unithiol) led to a reduction of the oxygen pressure in the tissues. With respect to cystamine, the data are less clear.

These polarographic studies, however, do not provide any basis for a categoric negation of a connection between the protective effect of thiol compounds and the oxygen effect, which was correctly pointed out by the investigators themselves. In particular, the current methods of polarographic analysis reveal only /97 the total oxygen balance in the tissue, and do not make it possible to measure the oxygen pressure directly in the cellular organelles, which is of critical significance in the decrease of the radiosensitivity. This is indicated by the fact that an increase in the oxygen pressure does not completely do away with the protective effect of biogenic amines (serotonin, histamine, adrenalin), although the oxygen pressure in the tissues even exceeded the normal level [7, 8].

The "pharmacological" hypoxia produced by indolylalkylamines (as a consequence of their vasoconstrictive effect) lasts several hours, while the maximum protective effect of these preparations develops 5 - 30 minutes after administration [35].

Data have recently been obtained on protection by means of indolylalkylamines both of solutions of polymers and isolated cells. Data up to 1964 have been collected in a monograph by Bak [2]. Among the new factors, let us point out the results obtained by N. P. Dubinin and L. G. Dubinina [9], concerning the reduction of the number of chromosomal aberrations in a culture of

fibroblasts during protection by serotonin, the protection of planaria by serotonin and catalase in solution. These facts indicate that other mechanisms of action of serotonin are possible, besides the "pharmacological" hypoxia.

In addition, a theory exists that concurrent relationships exist between the oxygen and the protective substances as a result of which the protectors are able to attenuate the irreversible radiation damage activated by the oxygen, as is proposed in accordance with the concepts strongly developed in recent years by Alper concerning the "direct" oxygen effect. In this view, an important role is played in the protection process by the relative distribution of the preparations and oxygen in the microstructures of the cell.

According to the data of Jameson and Brenk, thiol compounds (MEA and AET) and dimethylsulfoxide do not produce an intracellular decrease of oxygen pressure. Serotonin produced only a slight decrease in pressure in intracellular structures, which cannot explain the mechanism of radioprotective action [33].

Protection by Influencing Metabolic Processes

Many examples of the influence of protectors on the course of energy and plastic metabolism have been described: oxidation and oxidation phosphorylization, metabolism of nucleic acids, etc. [2]. However, the concept that the most effective antiradiation substances, for example, aminothiols, exert a prophylactic effect due to intervention in the metabolism of substances in the /98
organism has not received sufficient experimental confirmation.

Taking into account the considerable differences in the radiosensitivity of organisms of different species, sexes,

different tissues of the same organism, and finally the stages of the life cycle of the cell, E. Ya. Grayevskiy [7] feels that the factors which change the metabolic processes may have an influence on the radiosensitivity.

A. S. Mozzhukhin and F. Yu. Rachinskiy [15] feel that it is possible to link normalization of nucleic metabolism to protection of the cells of the marrow by cysteamine during the period immediately following irradiation, mentioned by P. G. Zherebchenko et al. [12, 13, 14] and Langendorff and Hagen [34].

In this connection, a factor that is of particular interest is the antiradiation effect of the inhibitors of mitosis — colchicine* and its derivatives, calcium chloride and urethane when administered to rats 24 to 48 hours prior to exposure. The antiradiation effect of these substances lies in their intervention in a proliferation of cells of radiosensitive tissues.

The increase in radioresistance of biological objects under the influence of aminothiols and indolylalkylamines, on the basis of the data of E. Ya. Grayevskiy, is determined by the increase in the content of endogenic thiol groups in the tissues of the organism [7, 8].

Bacq proposed a new hypothesis to explain the protective effect of sulfurous radioprotectors. Bacq et al. feel that an explanation of the protective effect of sulfurous radioprotectors by anoxic changes is not in agreement with the data indicating that the protective effect does not depend on the concentration of oxygen in the tissues at the moment of exposure.

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*Editor's Note: This is an alkaloid obtained from Colchicum autumnale.

The principle of competition among free radicals as the basis of the protective effect is supported by Bacq, inasmuch as considerable suppression of free radical formation takes place only where the concentration of aminothiol protectors in solutions is thousands of times greater than the concentration which may exist when radioprotectors are administered in protective doses to the animals.

The hypothesis of Eld'yarn and Pil concerning protection by the formation of protein hydrosulfide complexes contradicts the existence of correlations between the concentration of bonded sulfhydryl groups and the degree of the protective effect.

Bacq et al. suggest that the basis of the radioprotective effect of aminothiols is a process which Bacq has called "biochemical shock". The first stages of this process consist in the combination of aminothiols with proteins of cell membranes, changes in the permeability of the mitochondria and their rapid swelling and suppression of respiratory processes in the mitochondria as a result of disruption of carbohydrate metabolism. In addition, aminothiols inhibit the synthesis of thymidine and thymidylkinase and RNA-polymerase, leading to a disruption of DNA and protein synthesis.

According to the data of Bacq, there is a satisfactory correlation between these metabolic changes and the magnitude of the radioprotective effect. However, Braun and Koch [29] found that not only the aminothiols which possess the radioprotective effect, but also the compounds which are not active in this respect with sulfhydryl groups, produce disruption of the structure and swelling of the mitochondria. /99

In summarizing this brief review of the literature on protection and metabolic processes, we should point out that no clear parallelism has been established between the influence of radioprotective substances on metabolism and their antiradiation activity.

Protection and Recovery

It is obvious that irreversible damage to the cell arises when there are changes in the vitally important or irreplaceable molecules. It is most likely that such changes take place in the cell nuclei of radiosensitive tissues, although radiation evidently damages both the nucleus and the cytoplasm to equal degrees. It is possible that in the organism damage to the nucleus cannot occur without damage to the cytoplasmatic structures, which may in turn intensify the damage to the nucleus. However, changes in the cytoplasm during irradiation are less important for the cell and the entire organism, since the processes of recovery develop in it more rapidly and proceed during shorter times. Their cells can compensate to some extent, but the nuclear damage may lead to permanent genetic changes [19]. The maximum decrease in the biological effect of the dose, established on the basis of mammal mortality (LD_{50}) for the most effective radioprotective substances during radiation, only approximates the radioprotective effect obtained with hypoxia.

We can assume that, by reducing the irreversible portion of the radiation damage, the disruption of the functions of the individual organs and systems will act to a lesser degree on the condition of the entire organism. More suitable conditions are thereby produced for recovery of damaged structures at the expense of undamaged structures which are protected by the protector.

We know that in many cases the effect of protective substances takes the form of an attenuation of the damage to individual or certain systems. Preparations which exercise protective action, during irradiation at only the absolute minimum lethal dose, protect the hemopoietic system in particular (indolylakylamines, cyanides, etc.).

When the radiation dose is increased, the damage to the gastrointestinal tract is more severe. In this case, the protective effect is exercised by substances which are capable of protecting the gastrointestinal tract against radiation damage (AET, cystamine, tsistafos).

On the basis of observations on radiation damage, the opinion has become widespread that the effect of radioprotective agents does not have a significant influence on the damage to certain particularly radiosensitive systems of the organism, but leads to a retention of the hypothetical regenerative mechanism which promotes an earlier beginning and end of recovery processes in protected animals.

Experimental investigations in recent years have shown the error of this concept. On the basis of an analysis of experimental material obtained by the use of quantitative criteria, S. P. Yarmonenko [26, 27], E. Ya. Grayveskiy [7], Straub and Patt [39] and others have shown with sufficient certainty that antiradiation substances act by reducing the original damage of the tissues. The accelerated progress of recovery processes is possible because protective substances insure the preservation in an undamaged state of a great many cells, and attenuation of their initial damage, so that a more rapid and complete recovery of the damaged tissues is ensured.

These data show that the mechanisms of the radioprotective effect of the protectors are closely related to the compensation, repair and recovery of the affected physiological functions and the morphological substrate of the radiation damage.

Problems of Practical Utilization of Antiradiation Substances

Successes achieved in the experimental prophylaxis of radiation damage in various species of animals, including dogs and monkeys, have served as a basis for their use for prophylaxis of radiation complications which arise in radio- or x-ray therapy of malignant neoplasms in the clinic.

Positive results were obtained in tests of cysteine and cysteamine. The favorable effect of these substances on the symptoms of radiation damage has been demonstrated, and has made it possible to lengthen the course of x-ray therapy of malignant neoplasms [14, 27].

To a lesser degree than other effective radioprotective substances, there have been clinical studies of S-, β-aminoethylisothiuronium (AET, amituron). When this preparation is administered in doses of 400, 600 and 800 mg prior to x-ray or radium treatment, positive effects are observed. Due to a toxic effect, it was cancelled 10 days after the start. The author concluded that in patients who received the calculated radiation therapy at a dose of 11,000 to 13,000 r, the prophylactic use of 0.4 grams of AET 1 - 3 times a day is withstood satisfactorily and in two-thirds of the patients is not accompanied by primary radiation reactions. /101

The ability of healthy and sick people to withstand radioprotective substances has been studied by a number of authors. The results of these investigations are presented in many works [15, 17]. It has been shown that cystamine in healthy individuals is withstood well at a dose of 600 - 1200 mg/kg without any significant changes in the indices of respiration, hemodynamics and blood picture. AET is withstood less well by healthy and sick people than cystamine, with a toxic effect in approximately 50% of those investigated.

In conclusion, it should be mentioned that, in addition to the practical significance, a modification of the radioprotective effect is extremely important for increasing the depth of our knowledge about the biological action of ionizing radiation and the initial effects of radiation damage [26].

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Pharmacological Properties of Substances for Medicinal Prophylaxis of Radiation Damage

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ABSTRACT. This survey presents data on the pharmacological properties of the principal and most effective radioprotectors: the aminothiols and the indolylalkylamines.

The question of the toxicity of protective substances and the effective doses, behavior and physiological effect of these substances are discussed.

This survey presents data on the pharmacological properties /102
of the principal and most effective radioprotectors: aminothiols and indolylalkylamines. Works on the radioprotective effect of nucleic acids and hypotheses regarding the mechanism of their action have been discussed in detail in a monograph by Ye. F. Romantsev [15]. For no group of protectors do we have at the present time any clear criteria for defining the physiological reactions which arise upon the introduction of radioprotectors, the necessary factors for protection, and side effects which have /103
no influence on the protective effect or attenuate it.

It is likely that the majority of vegetative reactions have nothing to do with the protective effect of the protectors, but we cannot fail to take the fact into account that they are always observed in those cases when radioprotectors have a protective

effect. In using small doses of preparations which do not lead to noticeable physiological changes, there is a sharp attenuation or complete disappearance of the protective effect [16].

In studying the action of radioprotectors, an item of the greatest interest is the reactions which take place during the first 30 - 60 minutes following administration of the preparation, inasmuch as the majority of known protectors exercise their protective effect precisely during this time. For a deeper analysis of the toxic effect of the protectors, it is necessary to study the results of their long-term effects.

Toxicity of Radioprotectors and Protective Doses

Data on general toxicity, obtained by various authors, often do not agree. These differences may be related to the age and strain of the animals used in the experiments.

β-Mercaptoethylamine (MEA). Various salts of mercaptoethylamine differ markedly in toxicity. All of the salts of MEA when administered intravenously or intraperitoneally in toxic doses lead to a general excitation, increased respiration and clonic spasms. Ten to 30 minutes later adynamia sets in, with difficulty in breathing; in the case of dogs and cats, there is profuse salivation and frequent vomiting. Death ensues when respiration stops.

Inasmuch as the clinical application of the preparation requires that it be given periodically, it is interesting to study the results of chronic application of MEA. Out of 50 mice which received 3 mg intraperitoneally (150 mg/kg) daily for 48 days, 48 survived. The animals withstood weekly adminstration of MEA

for more than 40 weeks. Spasms and death of individual rats followed intraperitoneal administration of 80 to 100 mg/kg of MEA-salicylate, 110-125 mg/kg of hydrochloride, 150-165 mg/kg of hydrobromide and nicotinate, 165 mg/kg of ascorbinate. In rabbits, MEA-salicylate when administered intravenously evoked spasms in a dose of 35-50 mg/kg. In the case of rats, the LD_{50} amounts to 232 mg/kg intraperitoneally. In the case of rabbits with intravenous administration, LD_{50} = 150 mg/kg [2]. The rabbits withstood daily intravenous administration of MEA in doses of 33 mg/kg for 15 days without visible changes while guinea pigs withstood a total dose of 693 mg/kg for 11 days.

β-Mercaptopropylamine (MPA) is less toxic in comparison to MEA. The LD_{50} for intraperitoneal administration in the case of mice is 850 mg/kg. The optimum protective dose is 500 mg/kg [15-41]. The most detailed information on the pharmacology of MPA is found in the monograph by Ye. F. Romantsev [15]. For intravenous administration, the LD_{50} for mice is 470 mg/kg. Administration of MPA chlorhydrate intravenously to mice, rats, and rabbits at a dose of 150 mg/kg does not produce visible changes in the behavior of the animals. At a dose of 200 mg/kg, there is apathy, increased respiration and development of tremor in individual animals. Normalization occurs in 1 1/2 to 3 hours.

Death of rats is observed at doses of approximately 400 mg/kg, and the sensitivity of young rats is almost the same as that of sexually mature ones. Repeated intraperitoneal administration of MPA in a dose of 200 mg/kg for five days did not produce significant changes in the behavior of the animals or in the blood picture.

The protective dose for rats in experiments of Ye. F. Romansev [15] with intraperitoneal administration was 300 mg/kg.

When administered internally, MPA does not exert a protective effect. The protective dose for dogs with subcutaneous administration is 100 mg/kg; LD_{50} is 125 mg/kg.

β-Aminoethylthiophosphoric acid. In the practical respect, it is interesting to study the low toxicity of the monosodium salt of β-aminoethylthiophosphoric acid. The LD_{50} for mice with intraperitoneal administration is 947 mg/kg. The ratio of the LD_{50} to the optimum radioprotective dose for this preparation is 2.65; for cystamine and MEA it is 2.23; and for AET, it is 2 [1].

Cystamine. The toxicity of cystamine and the protective doses used are approximately the same as for MEA. For mice, the LD_{50} is 214.5 mg/kg, for rats it is 126 ± 4 mg/kg with intraperitoneal injection, and 1035 mg/kg for oral administration. The protective doses are 150 mg/kg intraperitoneally for mice; for rats with oral administration, it is 600 mg/kg, and for mice it is 400 - 600 mg/kg [67].

Cysteine. LD_{50} for mice is 1.75 g/kg; the protective dose is 1.5 g/kg for intraperitoneal injection.

S,β-Aminoethylisothiuronium Br.Hbr (AET). The LD_{50} for intra-peritoneal administration of AET to mice is 170 - 500 mg/kg, depending on the strain and age of the mice [21, 31].

A comparison of the toxicity of AET for animals of different species with different modes of administration of the preparation was performed by Kelly et al. [31]. In the case of female rats, the LD_{50} was 302 ± 14 mg/kg intraperitoneally, and 685 ± 96 mg for oral administration of the preparation.

Dogs, even with rapid intravenous injection, are able to withstand as high a dose as 125 mg/kg. This dose is protective for dogs. The ability to withstand AET can be increased by administering a preparation to the animals intraperitoneally in increasing aomunts each day. For rats, LD_{50} is 288 mg/kg. With fractional administration of AET at intervals of 30 or 75 minutes, the LD_{50} for rats increased to 325 mg/kg. In rabbits, the phenomenon of adjustment did not develop (LD_{50} - 236 mg/kg). The daily /105 feeding to dogs of increasing doses of AET led in 10 - 12 days to an increase in tolerance of the preparation, and the value of LD_{50} for a single administration was 113 mg/kg (intraperitoneally); following "accommodation" — 160 mg/kg.

In dogs, toxic but nonlethal doses of AET lead to flow of saliva and tears, opistotonus, dilation of the pupils, contraction of the nicitating membrane, vomiting and defecation. Five minutes following administration of the preparation, these symptoms are replaced by a picture of general depression. During the 20^{th} to 60^{th} minutes, there is a slight anesthetizing effect. The toxicity of AET increases when it is administered 15 minutes after atropine, glucose or ethanol. Injections of aminazine and barbiturates had no effect on the survival rate of mice which had received lethal doses of AET.

In the case of dogs, the investigator succeeded in reducing vomiting through oral administration of pyridoxine together with AET (Vitamin B_6).

Vomiting in dogs started when cystamine was administered through a fistula in the small intestine. Use of this preparation with coating substances, locally anesthetizing substances, and in capsules did not prevent its emetic effects. Monkeys withstood

much higher amounts of cystamine and AET without vomiting. Administration of the substance through a stomach tube in amounts up to 400 mg/kg of cystamine and up to 125 mg/kg of AET did not cause vomiting in monkeys, but the administration of small quantities of preparation on the mucous membranes of the mouth caused immediate vomiting [13].

Evidently we must agree with the opinion of A. S. Mozzhukhin and F. Yu. Rachinskiy [13], P. P. Saksonov [16] and other authors that the emetic effect of sulfur compounds is caused by both the central and reflex influences, associated with the stimulating effect on the mucous membrane of the gastrointestinal tract. The rapid development of vomiting following intravenous injection (in 1 - 3 minutes) indicates the considerable significance of the central mechanism of the emetic effect in this connection.

Aminazine causes spasms in toxic doses of MEA, cystamine and AET, reduces vomiting in dogs and cats, and simultaneously increases by 35% the radioprotective effect of aminothiol protectors. P. P. Saksonov [16] has shown that lobeline, cytitone, corazol, Coramine, strychnine, caffeine, atropine, pachycarpine, a number of parasympathomimetic and sympathomimetic preparations, anesthetics, vitamins, hormones, compounds producing metahemoglobin and antihistaminic substances in therapeutic doses do not have any effect on the tolerance and toxicity of MEA. Sodium salicylate increased the toxicity. Adrenalin, sympatol, phenamine, glucose, urethane, hexenal, chloral hydrate and histamine produced an insignificant weakening of the spasms produced by MEA, but did not reduce the mortality. The attenuation of the toxicity of MEA was caused by sodium nicotinate, nicotinic and ascorbic acid, sodium bromide, aminazine, phenatine, spermine and pyridoxine. Hence, the possi- /106
bility of decreasing the toxic effect of radioprotective

preparations without reducing their antiradiation properties has been demonstrated.

Sulfurous protectors have a locally stimulating and necrotic-inflammatory effect. Tsistafos (sodium salt of β-aminoethylisophosphoric acid), when used locally, does not have a stimulating effect [1].

The LD_{50} for cystamine in irradiated rats is 740 mg/kg; for nonirradiated animals, it is 1250 mg/kg. The LD_{50} for AET when administered orally for intact rats is 1000 mg/kg, while for irradiated animals it is 300 - 150 mg/kg, depending on the radiation dose [21].

Dimethylsulfoxide (DMSO). Of the approximately 500 compounds presently known which have radioprotective action, only DMSO possesses the ability to exert a protective effect when applied to the surface of the body. With intraperitoneal administration of 10 g/kg of DMSO, 83% of irradiated mice survived 30 days; at 12.5 g/kg — 80%; at 5 g/kg — 57%; with surface application of DMSO, 67% survived.

Moos and Kim [37] found that submerging the tails of mice in DMSO for 5 - 10 minutes prior to irradiation was sufficient to produce a protective effect; in a dose range of 700 - 950 r, the value of the DRF* was found to be about 1.35.

Monkeys withstood 5 g/kg of DMSO given orally for 100 days without special disturbances [37].

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* Editor's Note: Dose reduction factor.

Indolylalkylamines (serotonin and 5-MOT). With intravenous administration of 5-MOT to white mice in a dose from 50 - 125 mg/kg and serotonin in a dose of 60 - 80 mg/kg, there was slight, brief disturbance of the animals, tremors and clonic-chronic spasms. The LD_{50} for 5-MOT is 102.5 mg/kg and 81 mg/kg for serotonin [12].

With subcutaneous and oral administration, there is much in common between the action of serotonin and 5-MOT. Beginning at a dose of 100 mg/kg, the preparations cause inhibition, ataxia, tremor and diarrhea. At 200 - 400 mg/kg, the same disturbances were more pronounced and positive. The original state was restored in two hours on the average. Increasing the dose to 600 - 800 mg/kg led to death of some of the mice. The LD_{50} for subcutaneous administration in the case of 5-MOT was 620 mg/kg; for serotonin it was 601 mg/kg. When administered orally, the LD_{50} for 5-MOT was 580 mg/kg.

The rats were more sensitive to the 5-MOT. The initial changes in the general condition (ataxia, slightly pronounced diarrhea) developed with intravenous injection of 5-MOT at a dose of 10 - 20 mg/kg. Above 30 mg/kg, there were brief chronic-tonic spasms, ataxia and diarrhea, and the animals lay down on their sides. The original condition was resumed in 20 - 60 minutes. With intravenous injection, death of the animals occurred at a dose of 50 mg/kg. Inhibition, ataxia and diarrhea were observed in rats with subcutaneous injection of 50 mg/kg and oral administration of 100 mg/kg.

For cats, the lethal dose with intravenous injection was 2.5 - 5 mg/kg. The toxicity of 5-MOT for mice changed with age [6]. /107

Newborn mice were almost unprotected by 5-MOT. The maximum protective effect is found in the later periods of postnatal development. The most effective protective doses for mice are 50 and 75 mg/kg parenterally, with survival rates of 77 and 85%, respectively [11]. The maximum protective effect of 5-MOT is obtained in a dose of 7.5 mg/kg given subcutaneously. Survival of the mice remained at the same level with an increase in the quantity of preparation administered up to 100 mg/kg.

Toxicity of Radioprotectors for Man

In monographs by Bacq[2], V. I. Kuznetsov and L. I. Tank [11], A. S. Mozzhukhin and F. Yu. Rachinskiy [13], P. P. Saksonov and others [16], information was collected on the ability of man to withstand aminothiol radioprotectors and the clinical use of this substance. On the basis of the data from Soviet authors, the doses which can be withstood by man when administered orally are 0.6 - 1.2 grams of cystamine and 0.4 - 0.6 g of AET.

The oral intake of cystamine at doses of 0.6 and 0.8 on one occasion, and three times a day with intervals of six hours, did not change the working ability of healthy individuals [11].

A study of the tolerance of AET by healthy individuals and sick people with various somatic ailments which had not been subjected to x-ray therapy, performed by V. I. Kuznetsov [11], showed that patients withstood the preparation less well. Following intake of AET, half of the patients showed painful sensations in the vicinity of the liver, fatigue, pyrosis and vomiting. When cystamine was taken, the number of complaints was much less.

There were no significant changes observed in the condition of the cardiovascular system or the blood picture when radioprotectors were taken at the doses indicated in the data of Soviet authors [13].

A comparison of the toxicity of various salts of MEA, performed by the same authors, showed that ascorbate and hydrochloride did not produce side effects at doses of 200 - 250 mg with multiple (two times a week) intravenous injections. Hydrobromide and nicotinate produce a number of side reactions, and it was necessary to stop clinical use of these salts of mercaptoethylamine. Although the doses of aminothiols, used in the clinic on the basis of 1 kg of weight, were many times less than those used in the experiments on animals; nevertheless, the data from both Soviet and foreign authors indicate that the use of aminothiols prior to x-ray and radiotherapy sessions prevents the development of radiation sickness to a certain degree [2, 13].

The administration of 50 mg of 5-MOT 30 - 40 minutes prior /10
to exposure reduced the development of a general reaction in half of the patients who were subjected to radiation therapy. It had no influence on the course of the local reactions. In one-fifth of the patients, we noticed side effects of the preparation: fatigue, vomiting, headache, dizziness, speeding up of the pulse, pain in the stomach, diarrhea.

Intake, Distribution and Metabolism of Radioprotectors

In the last three years, only a few new works have been published dealing with the distribution and metabolism of radioprotectors. Inasmuch as these works do not contain any basically

new data, we will limit ourselves to a brief discussion of the question.

As we know, the maximum protective effect of the protectors occurs several minutes later with parenteral (and in 20 - 40 minutes with oral) injection [2, 30]. This feature has a satisfactory explanation, since in many works it has been shown that absorption of aminothiol protectors when administered parenterally takes place rapidly (in ten minutes), while when the substances are administered orally, it proceeds slowly. In the case of rats, during the first four hours only 20% of the cystamine introduced into the stomach has been absorbed. In dogs, absorption takes place much more rapidly: 30 minutes later, a significant quantity of the preparation was found in the blood, and the maximum concentration in the blood was found after two hours, with nearly complete absorption in three hours. Similar data were obtained for AET and disulfidamercaptoethylguanidine. Absorption of AET from the stomach of a dog takes place more slowly than for cystamine.

When mercaptoethylguanidine and its disulfides are administered orally to mice, they are absorbed rapidly, and in 30 minutes the concentration of the preparation is sufficient for protective effects to be evidenced.

The distributions of cystamine, MEA, AET, 2-mercaptoethylguanidine and 2-mercaptopropylamine in the tissues are very similar. Maximum concentration of these preparations was observed in the liver, spleen, intestine, kidneys, marrow and in much smaller amounts in the muscles and the brain, with very small amounts in the testes [3, 38]. The study of the distribution of dimethylsulfoxide has shown that this protector is distributed

comparatively uniformly through the soft tissues; the concentration of DMSO in the hard tissues, bone and cartilage is many times less. Concentration of the preparation in the spleen with superficial application is seven times less than after injection. The relatively high concentration of the label in the spleen and intestine was retained 10 - 40 minutes after intraperitoneal injection. When labeled serotonin was administered to mice, the maximum concentration in the liver was found in five minutes. The rapid increase in the concentration of the label in the kidneys during the entire period of observation (up to the 40^{th} minute) is associated with the rapid excretion of serotonin and its metabolites. The liver, spleen, kidneys and intestine contained up to 85% of the radioactivity administered [43]. 5-MOT, unlike serotonin, /10
passes through the hematoencephalic barrier [36]. In development of the radioprotective effect, the important role is played not so much by the total accumulation of preparations in the tissue, as by the distribution of protectors within the cell [13, 21].

V. G. Vladimirov [3] did not find any important interspecies differences in the distribution of the preparation in the tissues of the liver and spleen of mice and rats. Betz et al. [23] showed that in the case of MEA the ratio of free and bound forms changed with time.

The distribution of AET, MEA, GED and β-mercaptopropylamine in the organism takes place approximately in the same fashion as for cystamine and MEA.

According to the hypothesis concerning mixed disulfides (Eld'yarn and Pil) the radioprotective effect of the protectors is determined by the nature of the bond of the protein molecules in the protected biological substrate and the molecules of the

aminothiol protector. Alexander and Bacq suggest that the protective effect of aminothiol protectors depends on the increase in the concentration of three sulfhydryl groups [2, 20]. At the present time, none of these hypotheses has been supported experimentally (Z. Bacq [6], Ye. F. Romantsev [15]). It seems to us that it is not possible to state, as Bacq does [2], that during the several minutes following administration the principal mass of the thiol protector is bound with the proteins. Although this assumption is absolutely logical and it is difficult to assume that highly active aminothiols (in the chemical respect) could not react thoroughly with tissue proteins, nevertheless both in the works cited above and in many works which are discussed in the monograph of Bak there are data according to which the major portion of the protector is in the free state during the first 10 - 15 minutes following parenteral administration.

The question of the nature of the link between the radioprotectors and the proteins has been dealt with intensively in recent years, mainly from the standpoint of the hypothesis concerning mixed disulfides [36, 40].

The protective activity of thiazolines, including 2-aminothiazoline (one of the intermediate products of transguanidization of AET) depends on the formation in aqueous solutions of compounds with a free SH-group: 2-mercaptoethylamine, 3-mercaptopropylamine and derivatives [19]. In the hydrolysis of β-aminoethylthiophosphoric acid, MEA is liberated [2]. Cystamine is reduced in the organism of mammals to MEA [2, 13]. The final product of the metabolism of MEA in the organism is taurine, which has an insignificant protective effect in large doses.

Hence, while the metabolism of sulfurous protectors has been comparatively thoroughly studied, only a few papers have been

devoted to the metabolism of indolylalkylamines in the organism. Obviously, a more detailed study of the metabolism of radioprotectors will make it possible to discover the intimate mechanisms of their physiological and radioprotective action.

Effect of Radioprotectors on the Cardiovascular System /110

Mundy and Heiffer [38] found that narcosis in dogs prevented vomiting and spasms, but did not change the reaction of the cardiovascular system to the administration of MEA at a dose of 100 mg/kg given intravenously. The nature of prolonged hypotension (pressure drop to 20 - 60 mm Hg) could not be changed by the action of atropine, diphenylhydramine and the cutting of the spinal cord at the level of the first cervical vertebra. This indicates, in the opinion of the authors, that the development of hypotension has nothing to do with the effect of MEA on the central and parasympathetic nervous systems and the liberation of histamine. The magnitude of cardiac output decreased sharply prior to the beginning of the drop of arterial pressure. The pressure increased in the inferior vena cava. Hypotension coincided in time with bradycardia and an increase in the hematocrit readings. The authors connect the development of hypotension with a decrease in the return of the blood to the heart.

In their next paper, the same authors found that certain antihistaminic preparations reduced the hypotensive action of MEA in dogs [38]. The reduction of the hypotensive effect of cystamine by antihistaminic preparations was demonstrated previously in papers by A. S. Mozzhukhin et al. [13]. According to the data of several authors, intravenous injection of MEA in small doses (5 - 40 mg/kg) had no effect on the arterial pressure in either

anesthetized or intact dogs and cats. Doses of 65 - 75 mg/kg led to a slight decrease in pressure, while 100 to 125 mg/kg led to a considerable decrease in pressure. However, in decerebrated cats, MEA in a dose of 25 - 30 mg/kg causes a pressure drop.

When cystamine was given to dogs at a dose of 30 mg/kg, the maximum arterial pressure dropped by 10 - 15 mm Hg. The lateral systolic, minimum and average dynamic pressure did not change. The magnitude of the hemodynamic stroke decreased by 10 - 15 mm Hg, and the frequency of heart contractions did not change. Recovery of arterial pressure to the initial level took place two hours later with subsequent decrease and return to normal only after 24 - 48 hours. The vascular tone of the elastic type did not change during one hour following administration of the preparation; the decrease in tone started 2 - 3 hours later with simultaneous decrease in the peripheral resistance and an increase in the systolic and minute blood volume by 10 - 15%. At doses of 10 - 40 mg/kg cystamine given intravenously increased the systolic volume of the heart by 10 - 75% without changing the pulse frequency. At a dose of 50 mg/kg, cystamine administered intravenously caused tachycardia and an increase in the minute volume of the blood by 20 - 50% in nonanesthetized dogs [15]. Cystamine and MEA at a dose of 75 mg/kg in rabbits, given intravenously, caused an expansion of the vessels of the lesser circulation.

According to the data of P. P. Saksonov et al. [16], intravenous administration of various salts of MEA to cats and rabbits (15 - 75 mg/kg) led to a drop in the arterial pressure by 10 - 15 mm Hg. The intravenous novocainization and decerebration of carotid sinuses had no influence on the hypotensive effect of the preparation. However, according to the data of A. S. Mozzhukhin [13], rabbits with cutting or blockage of the sinocarotid nerves

showed an increase in the hypotensive action of MEA. With perfusion of the carotid sinus and retention of intact reflex pathways, MEA produces pressure increase.

In studying the influence of atropinization on the hypotensive effect of MEA in experiments on rabbits and cats, contradictory results were obtained [2].

According to the data of the majority of authors, cystamine has a hypotensive effect when administered in significantly smaller doses in comparison with MEA. In nonanesthetized rabbits, MEA and cystamine cause only a brief decrease of pressure, with return to normal in 1 - 2 minutes [13]. MEA blocks the anotropic and chronotropic effect of adrenaline on the isolated auricle of the rabbit and has no effect on the sensitivity of the tissue of the auricle to calcium chloride.

When cystamine is administered to animals, the vessels of various parts of the body react differently. Dilation of the vessels in the isolated rabbit ear is observed at a concentration of MEA equal to 10^{-3} and in the coronary vessels of the isolated rabbit heart at concentrations of 10^{-4} - 10^{-5}.

According to the data of other authors [16], AET in concentrations of 10^{-6}, 10^{-5} and 10^{-4} did not change the lumina of the vessels in the isolated rabbit ear, but reduced the vascular spasms caused by adrenalin.

According to the data of V. A. Kozlov and V. S. Shaskov [8], cystamine in a dose of 45 mg/kg given intraperitoneally to guinea pigs speeded up the heart rhythm, while at doses of 125 - 150 mg/kg it led to a sharp slowdown. Tsistafos in a dose of 150 mg/kg led to less pronounced and delayed decrease of rhythm.

Inasmuch as AET did not change the heart rhythm in these experiments, the authors suggest that the influence on the function of the heart is exerted by the presence of free amino and sulfhydryl groups. The presence of only one free amino or sulfhydryl group is insufficient to explain the effect. Atropine reduced the effect of cystamine on the heart, and dimedrol decreased (while pilocarpine increased) the effect of cystamine. Against the background of adrenalin, cystamine and MPA at a dose of 150 mg/kg led to the usual decrease in the frequency of the heart rhythm. The authors conclude that cystamine acts as a cholinomimetic.

According to the data of V. A. Kozlov [7], β-mercaptopropylamine, when given intravenously to rabbits at a dose of 30 mg/kg, leads to a brief decrease in the arterial pressure; when given to cats in the same dose, with injection of MPA, the pressure initially decreases and then increases. The depressor effect is done away /112
with by preliminary atropinization, while the pressor effect is reduced by cutting the vagus.

The intravenous administration of 150 mg/kg AET to dogs led to an increase in arterial pressure, as the result (probably) of the increase of the peripheral resistance. Following injection of AET, the myocardium did not react to stimulation of the vagus. Administration of atropine does not change the reaction of the cardiovascular system to AET. When AET is given orally at a dose of 30 mg/kg, the arterial pressure in dogs increases [32].

In cats under thiopental anesthesia, the intravenous administration of AET at a dose of 5 mg/kg caused brief decrease of pressure and inhibition of respiration. With an increase of the dose to 150 mg/kg, the cats died showing signs of a sharp decrease in pressure and cessation of respiration. Subcutaneous administration of AET in the same dose did not lead to significant

changes in the pressure and respiration. Preliminary administration of atropine reduced the hypotensive effect of AET; cytisine and hexonium did not change the reaction to AET. Against the background of action of AET, the hypotensive effect was retained, produced by stimulation of the preganglionic fiber of the vagus nerve. Similar results were obtained by Di Stefano et al. [26]. After the vagus nerves were cut at the neck, AET did not produce cessation of respiration and bradycardia, and the hypotensive reaction was completely prevented in all cases.

Preliminary administration of atropine prevented or reduced only hypotension and bradycardia but not apnea [14], as in the experiments of Di Stefano [26].

A drop in arterial pressure in rabbits caused by AET was accomplished when it was given in doses of 1 - 40 mg/kg.

In mice, the intravenous administration of a dose of 8 mg/kg of AET led to a slight biphasal change in the arterial pressure. Bradycardia and apnea were not observed, even when relatively high doses of AET were administered intravenously or intraperitoneally.

In the opinion of Di Stefano and others [26], radioprotectors in mice produce at least one pharmacological effect, which correlates with the protective effects, e.g., hypertension. From 1 to 1-1/2 hours following intravenous administration of cystamine in dogs, the plasma volume drops while the erythrocyte volume increases. The quantity of circulating blood drops to a lesser degree than the plasma.

Under the influence of AET, the volume of circulating blood increases. An increase in the hematocrit readings (thickening of

the blood) following administration of MEA and cystamine was observed by a number of authors [38].

The effect of radioprotectors on the permeability of the vessels in nonirradiated animals was studied in only a few papers. Following intravenous administration of cystamine in doses of 50 and 100 mg/kg in rabbits in the course of the first 1-1/2 to two hours, the authors observed an increase in the permeability of the hematoophthalmic barrier to fluorescein and protein. The /113 thickening of the plasma in dogs is accompanied by loss of plasma (together with protein contained in it) from the vascular canal.

The lymphatic vessels expand under the influence of aminothiols, and the lymph flow increases in them. Data from clinical studies on the action of aminothiols on blood circulation can be found in the monograph of V. I. Kuznetsov and L. I. Tank [11].

It is probably too soon to draw any kind of conclusion regarding the mechanism of the action of aminothiols on the cardiovascular system; there are too many contradictory experimental results.

Obviously, many of the contradictions have to do with the usual phasality of the effect which is associated with practically all pharmacological agents and the dependence of activity and intensity of individual phases on the dose of the preparation. A. S. Mozzhukhin and F. Yu. Rachinskiy [13] suggest that for small doses of aminothiols it is characteristic to have direct action on the vessels; at moderate doses, the direct action is insignificant and reflex reactions predominate from the carotid sinus to the vagus nerves, leading to hypotension, or to sympathetic innervation, which leads to hypertension. At large subtoxic doses,

such factors as the direct action on the vasomotor center, liberation of histamine and catecholamines and an increase in the sensitivity of the chemoreceptors may be significant.

Serotonin, unlike the catecholamines, changes the general resistance insignificantly, increasing it in large vessels and decreasing it in small ones. The vasoconstrictive effects of serotonin in the veins are intensified by catecholamines [30]. Histamine also causes narrowing of the arteries and veins and expands the small vessels (capillaries, arterioles and venules). 5-MOT, when given intravenously to anesthetized cats in doses of 0.5 - 1 mg/kg, has a pressor effect with a subsequent drop in pressure by 60 - 80 mm Hg [12]. The vasoconstrictor effect of 5-MOT has been insufficiently studied. The vessels of the isolated ears and kidneys of the rabbit are found to be narrowed by 5-MOT to approximately the same degree as by serotonin [12].

According to the data of L. F. Semenov [17], when various antiradiation amines are given in conjunction with acetylcholine in acute experiments on rabbits, monkeys and cats, the parasympathetic effects predominate (in the opinion of the author): a decrease in arterial pressure, speeding up of respiration, increase in amplitude of respiratory movements, and bradycardia.

The hemopoeitic organs have been studied under the action of radioprotectors in only a few papers. Due to the lack of experimental data, it is difficult to determine what causes the drop in oxygen pressure in the tissues: the disruption of the blood supply to the organs or the direct effect of the protectors on the enzyme systems.

M. D. Mashkovskiy and V. P. Lapskiy [12] found that under /114
the influence of 5-MOT the oxygen pressure in the brain decreases even when the blood flow to the brain increases.

Attempts to find a correlation between the radioprotective effect of protectors and their influence on the cardiovascular system, in our opinion, must begin with a careful study of the blood supply to radiosensitive organs and tissues.

At the same time, it is a matter of practical interest to find those pharmacological substances which reduce the toxic effect of protectors on the cardiovascular system. While the protective effect of indolylalkylamines and biogenic amines is determined by their ability to narrow the blood vessels of radiosensitive organs, thereby reducing the oxygen pressure in them, the problem of reducing the toxicity of these preparations consists primarily in the retention of normal coronary and cerebral blood flow, prevention of bronchial spasms and a reduction of the blood supply to the radiosensitive organs.

Influence of Radioprotectors on the Function of External Respiration

The death of animals following administration of lethal doses of radioprotectors, according to the majority of authors, takes place as the result of cessation of respiration. Inasmuch as in the experiments of Mandi and others prolonged artificial respiration did not always save the dogs when lethal doses of MEA were given [38], V. N. Kuznetsov and L. I. Tank suggest that the death of the animals, when poisoned with MEA, was a consequence not only of paralysis of the respiratory center, but of disruptions of hemodynamics as well [11].

Stimulation of respiration by intravenous administration of cystamine in anesthetized dogs at a dose of 60 mg/kg was recorded by A. S. Mozzhukhin [13]. At doses of 1.5 and 10 mg/kg, cystamine stimulates respiration of anesthetized cats. A. S. Mozzhukhin found that in unanesthetized rabbits cystamine at a dose of 20 - 50 mg/kg caused a speeding up and reduction of depth of respiratory movements [13]. In some animals, cessation of respiration set in for 5 to 15 seconds. Bilateral pharmacological extirpation or denervation of carotid sinuses blocked the effect of cystamine on respiration; cutting the vagus nerves did not change the reaction.

Novocainization and denervation of carotid sinuses have no influence on the reaction of respiration to MEA. It was shown in cats, depending on the dose of MEA, it is possible to produce both inhibition and stimulation of respiration. The effect of MEA on respiration takes place both through a reflex pathway and by means of direct stimulation of the respiratory center.

The administration of AET to anesthetized cats in a dose of 5 mg/kg intravenously leads to inhibition of respiration. According to the data of Di Stefano and others, AET in a dose of 2.5 /115
mg/kg in anesthetized cats led to apnea. Cutting the vagus nerves did away with the effect of AET on respiration; atropine did not prevent cessation of breathing [26].

Absorption of oxygen by anesthetized dogs [35] increased slightly when AET was given at a dose of 3.5 mg/kg. Intravenous administration of MEA to dogs at a dose of 100 mg/kg leads to a sharp drop in the saturation of the blood with oxygen. The oxyhemoglobin content in the arterial blood dropped to 50 - 60%, while that in the venous blood dropped to 25% [38]. The drop in

the oxygen content in the venous blood of rats when MEA and MPA were given in radioprotective doses begins (according to the data of Ye. F. Romantsev [15]) only after 25 - 50 minutes. Cystamine given to rats in a dose of 100 mg/kg led to a decrease in oxygenation of venous blood (from 12.7 to 4.4 relative %), while in the arterial blood the content of oxygen increased from 17.8 to 28.5 relative %. MEA at a dose of 125 mg/kg had no effect on the oxygenation of the blood. The increase in the oxygen content of the arterial blood was linked by the authors with a significant increase in the content of hemoglobin as a consequence of thickening of the blood. The decrease in the saturation of venous blood with oxygen in rats following administration of cystamine was demonstrated by Bacq [2]. A decrease in the oxygen requirement when aminothiols are given (AET, MPA, MEA, and cystamine) has been demonstrated in many papers [15, 21].

Effect of Radioprotectors on the Nervous System

Data on the participation of the nervous system in the protective effect of radioprotectors are limited. Although quite a few papers have been published dealing with the study of functional changes in various branches of the nervous system following administration of protectors, there are almost no data on the correlation of these changes with the protective effect of the preparations.

Removal of the brain and the nearby subcortex in mice has no effect on the severity and outcome of radiation sickness and does not affect the effectiveness of radioprotectors. Cutting the spinal cord and the sympathetic branches sharply decreased the protective effect of 5-MOT and had no effect on the protective effect of cystamine in the irradiation of animals locally, below the cutting point.

Cystamine and MEA have a sedative effect [5]. The intravenous administration of MEA to anesthetized cats at doses from 0.5 to 1 mg/kg or more leads to a slowing of respiration and a decrease in the amplitude of respiratory movements all the way to apnea [12].

Apparently the decrease in the oxygen requirement is one of several effects which are common to all radioprotectors [2, 6]. All protectors except for the bacterial polysaccharides, when given in large doses, reduce the body temperature and cause a drop in the redox-potential of the tissues. /11

The problem of the condition of the oxidation-reduction processes in the organism with introduction of radioprotectors has been discussed in detail in a monograph by Ye. F . Romantsev [15]. MEA (at a dose of 100 mg/kg) increased the functional activity of the hypothalamic region and the ascending pathways of the reticular formation of the brain 10 - 15 minutes after administration.

Indolylalkylamines (serotonin and 5-MOT) cause stimulation of the neurons of the reticular formation of the brain and depression of the synaptic transmission of signals in the cerebral cortex. 5-MOT is an antagonist of the diethylamide of lysergic acid with respect to its influence on the conditioned reflex activity of animals. In small doses, MEA has a blocking effect on the upper cervical ganglion of the cat, and in large doses MEA stimulated the cells of the ganglion. 5-MOT considerably increased the activation reaction threshold, caused by stimulation of the sciatic nerve, and reduced or completely blocked the perception of light flashes. The influence of 5-MOT on the central nervous system takes the form of antagonism of this compound with substances with a stimulating effect. In mice, it reduces or completely

does away with the motor excitation and hyperthermy produced by phenamine. In experiments on cats, there is antagonism observed between 5-MOT and phenamine as far as the effect on the bioelectrical activity of the brain is concerned. However, serotonin did not show any antagonism with respect to phenamine [12].

AET has been used in the clinic in treating a number of nervous ailments. AET brings patients out of a state of coma, enables them to recover their functions in paresis and paralyses, and is effective in the treatment of trophic cutaneous ulcers.

Effect of Radioprotectors on the Function of Smooth-Muscle Organs

In experiments on a section of isolated intestine taken from guinea pigs and rats, MEA in a dilution of $1 - 2 \cdot 10^{-6}$ increases the tone and the amplitude of the contractions of the intestine. Atropine weakens the effect of MEA. In higher concentrations ($2 \cdot 10^{-5}$) the effect of MEA has a biphasal nature: following intensification, there is an inhibition of the motor activity of the intestine. Stimulation of the intestine, caused by histamine or barium chloride, was reduced by MEA at a dilution of 10^{-4}. Intravenous administration of MEA at a dose of 50 mg/kg administered to rabbits in observations in situ leads to inhibition of the motor function of the small intestine; in dogs and cats it leads to a mild stimulation. At a dose of 10 mg/kg in cats, the preparation initially produces a slight increase in tone and contractions of the intestine, followed by a weakening of the peristaltic movements [16].

Depending on the concentration of cystamine, the effect of /117
histamine and acetylcholine on the isolated intestine of the

guinea pig is either attenuated or intensified. It decreases the drop in tone of the intestine caused by adrenalin and also reduces the increase in peristalsis caused by pilocarpine. Cystamine inhibits and prevents an increase in the tone of the intestine caused by acetylcholine, arecholine, histamine and barium chloride [11]. In a dilution of 10^{-4} to 10^{-5}, cystamine produced a slight decrease in the tone of the intestine without inhibiting peristalsis.

MEA blocks the effect of adrenalin on the isolated uterus of the rabbit, but blocks only a portion of the α-adrenoreceptors.

AET in a concentration of $5 \cdot 10^{-4}$ increases the amplitude of the contractions of segments of the small intestine of the cat, while at concentrations of 10^{-4} it considerably increases the tone of the intestine.

The effect of AET on the intestine is reduced by atropine. Against the background of pilocarpine, AET in a concentration of 10^{-4} caused a gradual slowing down of the contractions and a reduction of the amplitude, but the tone of the intestine did not decrease. In experiments performed in situ on rabbits which had previously been given morphine, AET in a dose of 5 mg/kg increased the tone and intensified the amplitude of the contractions of the intestine. The change in the motor activity of the intestine produced by AET was not prevented by atropine and was not changed by barium chloride [16].

On the basis of x-ray observations, cystamine and MEA when administered to rats in radioprotective doses cause a sharp decrease in the movement of the contrasting material through the intestine. MEA, when administered intravenously to dogs at doses of 10 - 60 mg/kg, slows down the movement of the contrasting material along the gastrointestinal tract.

In monkeys, dogs and cats, cystamine and AET administered orally in large doses produce vomiting, while parenteral administration produces both vomiting and an increase in peristalsis.

5-MOT, like serotonin, causes contraction of the smooth muscles. In comparison with serotonin, 5-MOT is less active and acts on isolated rabbit intestines and the cornu of the uterus of the rat in concentrations 10 - 50 times greater.

I. G. Krasnykh and A. R. Mansurov [10] found that cystamine, AET, tsistafos and mexamine slow down the evacuation of the contrasting material from the stomach of the rat when administered in radioprotective doses, but — in contrast to the data of L. I. Tank — they found that the protectors produce prolonged spasms of the pylorus and the prepyloric portion of the stomach.

In small concentrations, cysteine, cystamine, mercaptoethanol and 2, 3-dithiopropanol (BAL) intensify the reaction of isolated segments of the iliac intestine of the guinea pig to "slowly reacting" kinins: bradykinin and callidine [22]. AET in small doses had no influence on the contraction of the intestine produced by kinins. In higher concentrations, all of the above-mentioned SH-compounds suppressed the reaction of the intestine to the kinins (bradykinin, callidine, angiotensin), which the authors /118 explain by inhibition of the enzyme kininase by the sulfhydryl groups.

Hence, the authors of earlier works dealing with the study of the effect of radioprotectors on the contractility of the intestine failed to take into account the entire complex of regulatory movements of the intestine.

The Endocrine System and Biogenic Amines Involved in the Administration of Radioprotectors

The changes in the functional effect of the adrenals on the introduction of radioprotectors are apparently a consequence of a nonspecific stressor reaction. The increase in the adrenalin concentration in the plasma of dogs begins about ten minutes after administration of MEA (125 mg/kg), and the concentration of glucose increases at the same time. The concentration of noradrenalin decreases ten minutes after administration of MEA, and increases when cystamine is injected. Thirty - sixty minutes following administration of these preparations, the content of noradrenalin in the plasma increases by a factor of 2 to 3, and that of adrenalin —— by a factor of 25 - 65. Stimulation of the cortical layer of the adrenals when radioprotectors are administered was also observed by L. F. Semenov [17].

A. A. Pykhtina and V. S. Shashkov [14] found that, after administration of radioprotective doses of 5-MOT to mice, the level of the sympathomimetic amines increased in the tissues of the brain, liver, spleen, small intestine and blood 30 - 60 minutes after administration of the preparation. Tsistafos produced a significantly lesser increase in the content of sympathomimetic amines, and only in the tissues of the small intestine and the blood.

The level of ascorbic acid in the adrenals decreased under the influence of cystamine and finam, while AET and tsistafos did not lead to any changes [14]. All of the aminothiol and amine protectors, including AET, reduced the concentration of ascorbic acid in the adrenals.

From 1 to 1-1/2 hours following administration of MEA to dogs (75 mg/kg), the content of 17-oxycorticosteroids in the plasma of the peripheral blood increased by 3 - 4 times. At the same time, an eosinopenic reaction developed. In hypophysectomized animals, MEA does not produce stimulation of the adrenal cortex. The protective capacity of MEA and antipheine decreases in adrenalectomized mice, and completely disappears in adrenalectomized rats [9].

Radioprotectors, both aminothiols and amines, suppress the function of the thyroid gland [17, 29, 44]. Under the influence of MEA and cystamine, there is a two to three-fold increase in the ability of the thyroid gland to concentrate iodine, but there is no increase in the synthesis of the thyroid hormones. Radioprotectors do not produce any changes in the content of serotonin in the blood and tissues [17].

Biochemical Reactions to the Administration of Radioprotectors /119

Several of the hypotheses which exist at the present time concerning the action of radioprotectors are based on known facts concerning the increase of concentration in animal tissues of sulfhydryl groups, inhibition of processes of oxidative phosphorylization and oxygen transfer on the administration of radioprotectors [2, 5, 4, 33].

An increase in the level of sulfhydryl groups in the tissues following administration of aminothiols was observed by many authors. The number of sulfhydryl groups in the spleen of experimental animals, according to the data of E. Ya. Grayevskiy et al. [4, 5, 13, 14] was three to four times higher than in the control animals, even if we assume that the protectors administered were

not inactivated. The increase in concentration of sulfhydryl and disulfide groups, though less significant, was observed when aminothiols were given. MEA and cystamine do not increase the level of tissue nonprotein sulfhydryl groups [42].

Acetylcholine, adrenalin and their combinations do not change the level of sulfhydryl groups in the tissues of mice [17]. Following injection of MEA into rats, there was an increase in the concentration of tissue enzymes: lactate-dehydrogenase, phosphogluconatedehydrogenase, malatedehydrogenase and β-glucuronidase. A remarkable increase in the concentration of these enzymes in the plasma was observed after 25 minutes, with the maximum observed in two hours, and normalization taking place after six hours following administration of the preparation. The authors suggest that MEA produces a reversible "cell shock" with disruption of the microstructure of the cell and permeability of the cell membranes. They suggest that the basis of the protective effect of aminothiols is to be found in the formation of a complex with metal ions in the active center of the enzymes. The mechanism of the protective effect of serotonin and other amines is similar [32, 33]. Hence, the majority of the pharmacological effects produced by aminothiols apparently have no direct relationship to the protective effect.

However, the protective effect of biogenic amines apparently is closely linked to their physiological (pharmacological) activity, but the vasoconstrictive effect of amines and their ability to evoke hypoxia are not the only factor which governs the protective action of the preparations.

In conclusion, we would like to emphasize that the extensive study of pharmacological properties of radioprotective substances and preparations in separate and combined application will

facilitate the discovery and production of new compounds which are less toxic and more effective.

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Antiradiation Effectiveness of Cystamine Under Various Conditions of Gamma Irradiation

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ABSTRACT. When cystamine is administered intraperitoneally to mice 5 to 10 minutes prior to gamma-irradiation (139 - 159 r/min), there is a linear dependence of the dose reduction factor (DRF) on the cystamine dose. The increase in the administration time of the preparation at 15 mg/kg to 30 minutes prior to irradiation does not have a significant influence on its protective properties. One hour later, the antiradiation activity of the protector decreases sharply. When cystamine was used in doses of 50 - 100 mg/kg, the decrease in the protector protective properties was noticed at earlier stages. With irradiation for 1.5 - 4 hours, the protective activity of cystamine, AET, tsistafos and 5-MOT decreased sharply.

Cysteamine and cystamine provide the best protection for mice and rats which have received an intraperitoneal administration of the preparation 5-10 minutes prior to exposure. Forty-five to 60 minutes later, the antiradiation properties of the radioprotectors decrease significatntly. To some extent, the relatively short period of optimum action of radioprotective preparations produces their low effectiveness in prolonged exposure [6, 11, 12, 13, 21, 25].

The degree to which the protective effect of the protectors is manifested and the duration of the optimum effect of the substances are determined in turn by the size of the dose given, as well as the dose of radiation. Patt et al. [29] observed that

at a constant dose of radiation the antiradiation effectiveness of cysteine is in direct relationship to the logarithm of the dose of the compound. This fact was confirmed later for other aminothiols as well [7, 22, 23].

In the case of indolylalkylamines, a different dose dependence is characteristic: following attainment of a certain level of antiradiation effectiveness, further increasing the protector dose has no significant influence on the protective activity of the compound — there is a plateau in the dose [7].

There is no uniform opinion among radiobiologists concerning the protective properties of radioprotectors after various doses of radiation. Many investigators feel that, as the dose of radiation increases, the relative effectiveness of the antiradiation compounds increases as well [17, 22, 23, 27]. However, other investigators do not support this fact [9, 16, 26, 28].

In conjunction with this fact, it was quite interesting to obtain more detailed characteristics of the antiradiation effect of the various doses of aminothiols (using the example of cystamine dichlorhydrate) at various doses of radiation, various intensities of radiation dosage and various periods of protector administration prior to irradiation under methodically comparable experimental conditions.

The experiments were performed on white random-bred mice, /12
weighing 20-25 grams, of both sexes. The animals were kept on an ordinary diet. The mice were exposed to gamma radiation on a GUBE-800 circular apparatus with a Co^{60} source (dose intensity 159-139 r/min) and on a "Khizotron" gamma-apparatus with a Co^{60} source at a dose intensity of 12.5-2.5 r/min. The animals were irradiated during the daytime at all seasons of the year. With

the GUBE-800 apparatus, the mice were irradiated in batches of 10 in cylindrical cages made of plastic, 95 mm in diameter and 90 mm high, while on the "Khizotron" they were exposed in batches of 10 in special cylindrical cages made of plastic with a compartment for each animal.

The preparations were dissolved in distilled water and administered to the mice intraperitoneally in a volume of 0.1 ml/10 grams of weight of the animal. The observations of the experimental mice were conducted over a period of 30 days. The radiobiological effect of the irradiation and the antiradiation effect of the preparation were evaluated on the basis of the clinical picture of radiation damage while the mortality of the animals during the 30 days after exposure, the average survival time of the animals which died (AST) and the dynamics of their weight following exposure were followed. The average lethal dose ($LD_{50/30}$) of the ionizing radiation for the experimental mice was determined by the method of sample analysis.

Antiradiation Effectiveness of Cystamine as a Function of the Preparation Dose

Cystamine in different doses (25, 50, 75, 100 and 150 mg/kg as a basis) were given to the mice intraperitoneally 5 to 10 minutes prior to exposure in doses of 850, 900 and 1000 r (dose intensity was 159-139 r/min). Cystamine in doses of 50 to 150 mg/kg reduced the severity of the radiation damage and the mortality of the experimental animals, but at a dose of 25 mg/kg it did not have any significant influence on the mortality of the mice during the 30 days following exposure (Table 1).

A dose of 50-150 mg/kg showed high antiradiation effectiveness against "the intestinal" form of death of the mice. Thus,

TABLE 1. ANTIRADIATION EFFECTIVENESS OF VARIOUS DOSES OF CYSTAMINE 5 to 10 MINUTES PRIOR TO GAMMA IRRADIATION (139-159 r/min).**

Dose, r	Dose of Cystamine, mk/kg	Number of mice	Survival, %		MAST, days
			5 days	30 days	
850	0	98	86,7±3,4	12,2±3,2	10,0±0,4
	150	79	100,0	93,6±2,7*	20,2±12,5
	100	58	98,3±1,7*	89,6±4,0*	12,3±2,6
	75	59	98,3±1,7*	64,4±6,3*	13,2±1,1*
	50	79	97,5±1,8*	46,5±5,6*	12,5±0,2*
	25	56	82,1±5,1	14,3±3,3	10,1±0,5
900	0	116	55,2±4,6	0,9±0,9	6,7±0,2
	150	47	100,0	91,5±4,3*	13,3±1,0*
	100	40	100,0	62,5±7,6*	15,2±1,5*
	75	60	93,4±3,2*	35,0±6,2*	11,7±1,0*
	50	60	90,0±3,9*	3,3±2,8	11,0±0,4*
	25	79	64,6±5,3	0	6,8±0,3
1000	0	39	41,0±7,9	0	6,5±0,4
	150	39	92,3±4,3*	71,8±7,2	12,5±1,8*
	100	36	91,7±4,6*	27,7±6,2	10,6±0,6*
	75	40	80,0±6,3*	20,0±6,6	9,9±0,8*
1100	0	20	0	0	3,9±0,3
	150	40	100,0	22,5±6,6	9,9±0,7*
1200	0	66	0	0	3,4±0,5
	150	49	55,1±7,1	0	7,1±0,5*
Control		40	100,0	100,0	--

* $p < 0.05$.

** Commas represent decimal points.

for example, the survival on the fifth day following irradiation (900 r) in the experimental groups was 80 to 100%, while in the control group it was only 55.2% ($p < 0.05$). Upon analyzing the survival rate and the changes in weight of the experimental mice during the 30 days, we observed a certain

relationship between the protective effect of the cystamine and the preparation dose (Figure 1). Thus, cystamine given in doses of 150, 75 and 50 mg/kg produced a survival rate of 92.5, 35.0 and 3.3% of the mice, respectively, with 99.2% mortality in the control group which was exposed (900 r). Consequently, the decrease in the optimum dose of cystamine by a factor of 2 to 3 led to a reliable decrease in the antiradiation effectiveness of the protector ($p < 0.05$). It is clear from Figure 1 that the weight of the mice in the control group on the third day following exposure (900 r) fell by 16.2%, by the 6^{th} day it fell by 21.1%, and by the 10^{th} day it fell by 22.5%. The decrease in weight of the experimental mice was evidenced to a lesser degree than in the control animals, and differently as different doses were given.

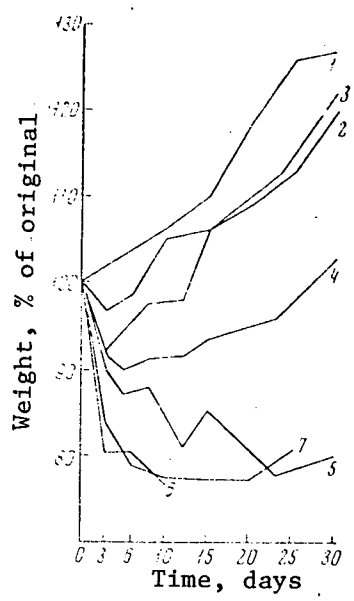

Figure 1. Dynamics of the change in weight (% of original) in mice during the 30 days following gamma irradiation at a dose of 900 r (139-159 r/min).

1 - control without irradiation; cystamine: 2 - 150; 3 - 100; 4 - 75; 5 - 50; 6 - 25 mg/kg; 7 - control with radiation.

Beginning with the 5th and 6th days following exposure, the groups with protection showed an increase in the weight of the mice. As we can see from Figure 1, the recovery of the original weight of the animal in groups with administration of cystamine at a dose of 150 mg/kg took place in seven days, at a dose of 100 mg/kg — 13 days, and at 75 mg/kg — 27 days after irradiation, respectively. /121

It should be pointed out, however, that even by the 30th day of the sickness the weight of the mice in the experimental groups was less than for the animals in the control group which had not been irradiated (see Figure 1).

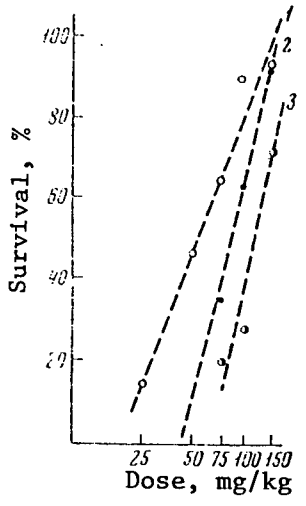

Figure 2. Survival of experimental mice 30 days after exposure to 850 r (1), 900 r (2), and 1000 r (3) with prophylactic use of cystamine.

Figure 2 shows the dependence of the antiradiation effect (on the basis of the survival rate of the mice by the 30th day after exposure) of cystamine on the protector dose at the same radiation dose. As we can see from Figure 2, the protective properties of cystamine bear a direct relationship to the logarithm of the dose of the preparation.

Radioprotective Activity of Cystamine at Various Doses of Radiation

/125

Cystamine at doses of 75, 100 and 150 mg/kg was administered to mice intraperitoneally 5 to 10 minutes prior to exposure. The animals were irradiated at doses of 500 to 1200 r (159-139 r/min).

The evaluation of the effectiveness of the cystamine was performed not only on the basis of the mortality indices, AST and the weight of the animals, but also on the dose reduction factor (DRF) of the protector. The results of these studies are shown in Table 1.

It is evident from the material presented that at superlethal doses of gamma radiation the protective effect of the cystamine decreases markedly. Thus, for example, irradiation at a dose of 1100 r cystamine (150 mg/kg) in 30 days led to survival of only 22.5% of the mice; at 1200 r in 5 days it protected 55.1% of the animals, but all of the experimental mice had died by the 30^{th} day.

The decrease in the percentage of "intestinal" death of experimental animals led to an increase in the AST of the mice by a factor of more than 2 ($p < 0.05$). Figure 3 shows the sample curves for the radiosensitivity of experimental mice with prophylactic use of various doses of cystamine as a function of the dose of radiation.

It is evident from Table 2 that the DRF of cystamine in doses of 150, 100 and 75 mg/kg is 1.49 (1.42 - 1.56), 1.33 (1.27 - 1.40) and 1.25 (1.19 - 1.31), respectively.

TABLE 2. ANTIRADIATION EFFECTIVENESS (ON THE BASIS OF DFR) OF VARIOUS DOSES OF CYSTAMINE, ADMINISTERED 15 - 10 MINUTES PRIOR TO IRRADIATION AT A DOSE LEVEL OF 139 - 159 r/min.

Dose, mg/kg	No. of Animals	LD_{50}, r and confidence intervals for p = 0.05	Function of slope of victim line	DRF and confidence intervals for p = 0.05
0	529	700.0 (671.8 - 729.4)	1.16 (1.1 - 1.23)	-
150	287	1040.0 (1004.8 - 1076.4)	1.12 (1.1 - 1.17)	1.49 (1.42 - 1.56)
100	144	933.3 (906.7 - 958.5)	1.17 (1.12 - 1.22)	1.33 (1.27 - 1.40)
75	159	873.0 (848.4 - 898.3)	1.14 (1.1 - 1.19)	1.25 (1.19 - 1.31)

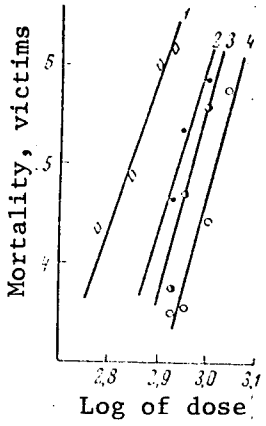

Figure 3. Mortality of experimental mice at various doses of radiation (139 - 159 r/min):
1 - control with irradiation; cystamine; 2 - 75; 3 - 100; 4 - 150 mg/kg.

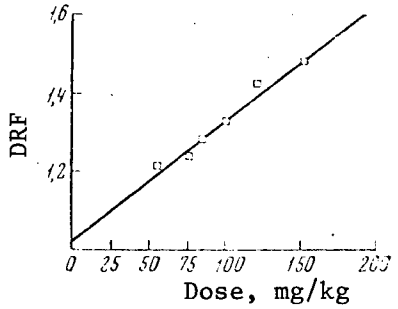

Figure 4. Antiradiation effectiveness of cystamine, determined by the dose reduction factor (DRF).

Figure 4 shows the dependence of the antiradiation effectiveness of the cystamine (on the basis of the DRF) on the protector dose. As we can see, when the radioprotective activity of the cystamine is expressed in the relative indicator (DRF), indicating the degree of radiation damage decrease, the dependence of the protector effect on the dose of preparation has a linear nature and may be described by a linear equation:

$$DRF_{cys} = 1.024 + 0.0031 \cdot D_{cys}, \text{ where D is the dose (mg/kg)} \quad (1)$$

However, as we noticed earlier (see Figure 2), at a given /126
radiation dose the radioprotective effect of cystamine is directly related to the logarithm of the dose of the material. Therefore, it is of particular interest to determine to what extent these two facts correspond to one another.

On the basis of the graphs in Figure 2, we calculated the doses of cystamine which can protect 50% of the mice when exposed to radiation at doses of 850, 900 and 1,000 r, and then the DRF of these doses of preparation ($LD_{50/30}$ for mice under these irradiation conditions, corresponding to 700 r). Hence, it was found that the DRF of cystamine at doses of 54.9, 85.1 and 117.5 mg/kg was equal to 1.21, 1.29 and 1.43, respectively.

These data were plotted on a graph showing the dependence of the antiradiation effectiveness (in DRF) of cystamine on the dose of material (Figure 4). As we can see from Figure 4, the DRF of the protector, calculated on the basis of the linear dependence of the effect on the logarithm of the preparation dose (see Figure 2) shows good agreement with the linear relationship described by Equation (1).

Hence, the linear dependence of the protective activity of cystamine on the logarithm of the substance dose (at a constant radiation dose) is apparently a partial variant of the dose dependence of the effect of aminothiols on the doses of preparation and radiation.

Antiradiation Effect of Cystamine at Various Times Following Application of the Preparation /127

At doses of 50, 100 and 150 mg/kg, cystamine was given intraperitoneally 30 and 60 minutes and 3, 5, 8 and 12 hours prior to gamma irradiation (900 r, 139 r/min).

As we know, the optimum period for the development of protective properties of cystamine with intraperitoneal administration is 5 to 10 minutes prior to irradiation. After 30 minutes the protector, given in a dose of 150 mg/kg, retains its high

antiradiation activity. However, 60 minutes later there is a sharp decrease in the effectiveness of the preparation. During the next five hours, the protective properties of cystamine remain at this same low level (Table 3). /128

After 8 and 12 hours, the protector does not have any antiradiation effect. When cystamine is used at a dose of 100 mg/kg, the decrease in the effectiveness of the protector takes place somewhat earlier — after 30 minutes (Figure 5). During the next five hours, cystamine produced approximately the same effect at this dose as at the optimum dose of the preparation.

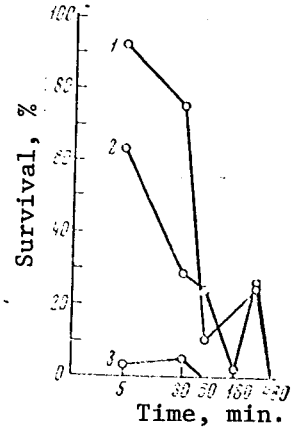

Figure 5. Survival of mice on the 30th day after exposure to a dose of 900 r (139 - 159 r/min) with prophylactic application of cystamine at various times prior to beginning of exposure.

1 - 150; 2 - 100 and 3 - 50 mg/kg.

At a dose of 50 mg/kg, cystamine displayed slight antiradiation properties 30 minutes following application of the substance, while after 60 minutes the protector did not show any protective effect at all.

Influence of the Radiation Dose Intensity on the Antiradiation Effectiveness of Cystamine

In this series of experiments, mice were irradiated for 1 1/2 and 3 to 4 hours. A comparison of the protective effect of the protector was performed at the absolutely minimum lethal dose of radiation which corresponded to 950 r at a dose intensity of 11.5 r/min, and to 1100 r at 4.6 - 5.2 r/min.

TABLE 3. ANTIRADIATION EFFECTIVENESS OF CYSTAMINE AT VARIOUS INTERVALS FOLLOWING ADMINISTRATION OF THE PREPARATION (900 r, 139 - 159 r/min).**

Time of administration of preparation prior to irradiation, hours	Dose, mg/kg	No. of mice	Survival, %		AST, days
			5 days	30 days	
Irradiated without protection	0	171	57,2±3,8	0,6±0,6	6,7±0,2
0,5	150	57	94,8±2,9*	75,4±5,7*	13,8±2,1*
	100	77	90,9±3,3*	28,6±5,1*	12,6±0,8*
	50	100	84,0±3,7*	5,0±2,2	9,9±0,5*
	25	40	72,5±7,1	0	7,7±0,4
1,0	150	29	96,6±3,4*	10,3±5,6	13,6±0,9*
	100	21	100,0	33,8±9,3*	13,8±4,1
	50	20	75,0±9,7	0	11,2±1,4*
	25	20	70,0±10,3	0	7,8±0,2
3,0	150	66	89,4±3,7*	19,2±4,9*	11,0±0,4*
	100	39	97,5±3,2*	2,6±2,2	11,0±0,4*
	50	20	85,0±8,0*	0	10,7±1,1*
	25	20	70,0±10,2	0	7,6±0,6
5,0	150	20	90,0±6,7*	25,0±9,7*	9,2±0,8*
	100	15	93,3±7,0*	26,6±11,4*	10,2±1,1*
8,0	150	19	80,0±8,9*	0	7,2±0,4
	100	20	75,0±9,7	0	6,4±0,5
	50	20	65,0±10,7	0	6,4±0,4
	25	20	70,0±10,2	0	7,1±0,6
12,0	150	20	90,0±6,7*	0	8,9±0,6*
	100	20	85,0±8,0*	0	7,7±0,5*
Control	0	40	100,0	100,0	—

* $p < 0.05$.

** Commas represent decimal points.

The radioprotectors were administered to the mice intraperitoneally, 10 - 15 minutes prior to exposure. Cystamine was used in doses of 25, 50, 75, 100 and 150 mg/kg; S-,β-aminoethylisothiuronium-bromide-hydrobromide (AET) was given in a dose of 150 mg/kg; the monosodium salt of β-aminoethylthiophosphoric acid (tsistafos) — in a dose of 300 mg/kg; and 5-methoxytryptamine (5-MOT) — in a dose of 75 mg/kg.

The results of the experiment are shown in Table 4. When the exposure time was increased to 1.5 hours, the antiradiation properties of the cystamine decreased considerably relative to /129
its effect at a dose intensity of 139 - 150 r/min (see Table 1). This was most clearly evident when the protector was used at doses lower than optimum. Thus, for example, cystamine at a dose of 100 mg/kg in the case of exposure for 5 - 7 minutes (dose of 900 r, 139 - 159 r/min) produced a 62.5% survival of the mice, while with exposure for 1.5 hours at the same dose only 10% of the animals were protected ($p < 0.05$).

Under conditions of gamma radiation for 3 - 4 hours, cystamine AET, tsistafos and 5-MOT had practically no radioprotective effect (Table 4). The material presented, dealing with the change of antiradiation effectiveness of cystamine in the optimum dose, depending on the period of application of the preparation prior to irradiation, confirms the data in the literature [1, 6, 11, 12, 13, 21, 25] on the sharp decrease in its protective properties /130
45 - 60 minutes following the parenteral application. These facts correlate positively with the experimental data on the content of cysteamine and cystamine in the tissues of the animals [8, 15, 31]. After 45 to 60 minutes, the radiosensitive tissues still contain 20 - 30% cystamine-cysteamine, while after 3 and 5 hours there is only 10 - 15% of the substance remaining [15, 31].

TABLE 4. ANTIRADIATION EFFECTIVENESS OF RADIOPROTECTORS WITH INTRAPERITONEAL ADMINISTRATION 10 - 15 MINUTES PRIOR TO EXPOSURE**

Dose and dose intensity of radiation, r/min	Preparation	Dose, mg/kg	No. of animals	Survival on the 30th day, %	AST, days
950 (10,5—11,5)	Cystamine	—	79	1,2±1,2	10,6±0,2
		150	58	51,7±6,6*	20,0±2,8*
		100	20	10,0±6,7	11,5±1,2
		75	39	7,7±4,3	10,3±0,7
		50	19	5,2±5,1	9,9±0,9
		25	19	0	9,1±0,5
1100 (4,6—5,2)	Cystamine	—	108	0	8,5±0,3
		150	99	3,1±1,7	10,8±0,3*
		100	18	5,5±5,3	10,3±2,3
	AET	50	40	0	9,1±0,4
		150	59	3,4±2,4	10,9±0,3*
	Mexamine	75	40	2,5±2,5	10,7±0,8*
	Tsistafos	300	59	1,7±1,6	9,5±0,3*
1200 (2,4—2,5)	Cystamine	—	19	0	9,8±1,3
		150	20	0	9,9±0,5
		100	20	0	8,6±0,5
		50	20	0	8,9±0,5
0	Control	0	40	100,0	—

* $p < 0,05$.

** Commas represent decimal points.

Nevertheless, the stabilization which is observed to a certain extent even with a low protective effect of cystamine during the subsequent 2 - 5 hours is difficult to explain by the pharmacokinetics of the substance. Bacq [12] also noticed a higher effectiveness of cystamine after four hours than after two hours following its internal application (optimum period —

30 - 60 minutes later). It is possible that this effect is caused by an aftereffect of the protector. P. G. Zherebchenko et al. [4] feel that in the second phase of the action of cystamine there may be involvement of the hypophyseal-adrenal system. The pronounced changes in metabolic processes of the organism, under the influence of aminothiols in particular, take the form of an increase in the tissues at the level of endogenic sulfhydryl groups [2, 30], and may play a certain role in the changed reactivity of the animals to irradiation at remote periods following the application of antiradiation compounds.

When using cystamine in doses lower than optimum, the decrease in the antiradiation properties of the preparation took place at earlier periods following administration of the protector. This effect is apparently due to a considerable degree to the low initial concentration of the substances in the tissues of the animal when small doses are administered.

The pronounced linear dependence of the protective activity of cystamine, on the basis of the DRF, on the preparation dose also supports the role of the protector concentration in the tissues of the organism. The linear nature of the dependence of the effect of aminothiols (AET) on the dose was observed earlier in experiments on bacteria and isolated cells [20] and in experiments on mice involving administration of various doses of AET and APT [18].

As indicated by our own experimental data in the test range of radiation doses (850 - 1200 r), the relative activity of cystamine did not change, which to some extent supports the data of many authors [9, 16, 26]. Nevertheless, on the basis of various tests to evaluate the effect of protectors, this dependence will evidently not be unambiguous [24]. This may partly be due to the

fact that there is different radiosensitivity in a number of critical systems (lymphoid tissue, small intestine, marrow) in the organism and different tropicity of certain radioprotectors with regard to these tissues.

The facts which we obtained concerning the antiradiation effectiveness of cystamine, AET, tsistafos, and mexamine in prolonged irradiation support the data of other investigators [5, 10, 14, 19] concerning the sharp decrease in the protective properties of protectors with irradiation lasting several hours. /131

Apparently, in addition to the pharmacokinetics of the protector, which causes corresponding changes of the effectiveness of the substance in time, the antiradiation properties of the radioprotective compounds with prolonged irradiation are influenced by the radiation itself. Under these conditions, it is possible to expect significant changes in the pharmacokinetics of antiradiation substances which apparently lead to a clear drop in the protective effectiveness of the protectors.

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Chemical Protection Against Mixed Gamma-Neutron Radiation With Various Contributions of Neutrons to the Absorbed Dose

/132

M. V. Vasin, V. A. Kozlov, B. L. Razgovorov and I. G. Krasnykh

ABSTRACT. Results are presented of studies on antiradiation effectiveness of cystamine, AET, tsistafos and 5-MOT in intraperitoneal administration to mice under conditions of mixed gamma-neutron irradiation with a neutron contribution to the absorbed dose of approximately 35 or 90% (average energy of the neutrons $\approx$ 1 MeV). Most of the damage in the symptom complex of radiation sickness in the mice with irradiation by neutrons was that of the intestine. Under conditions of mixed gamma-neutron irradiation with a neutron contribution to the absorbed dose of about 90%, AET and tsistafos slightly decreased the severity of radiation damage. The survival rate of the mice under these conditions was 24.2 (AET) and 26.8% (tsistafos) at $LD_{90/30}$. Cystamine and 5-MOT were ineffective. In joint use of AET and tsistafos with 5-MOT, the protective properties of the aminothiols did not increase. With a drop in the contribution of the neutron component to 35%, AET, cystamine and tsistafos produced remarkable antiradiation effects, while 5-MOT did not.

Chemical protection against neutron radiation is a poorly investigated problem. The very scarce data available in the literature indicate low effectiveness of radioprotectors against this form of radiation [7, 8, 9, 10, 13].

On the basis of the data of A. G. Sverdlov and others [4], when the neutron component in mixed gamma-neutron radiation is reduced to 25 - 30%, the protective effectiveness of AET (S-, β-aminoethylisothiuronium-bromide-hydrobromide) and cystamine appears to the same degree as under conditions of gamma or x-radiation.

The question of the effectiveness of pharmacochemical protection methods with an increasing dose of the neutron component has been a subject of our studies. The experiments were performed on white random-bred mice (females) weighing 19 - 22 grams, kept under normal conditions in a vivarium on a standard diet.

Irradiation of the animals was performed on a uranium research reactor with mixed gamma-neutron radiation. Two modes of exposure were employed. In the first mode of irradiation, the contribution of the neutrons to the absorbed dose (neutron component) was about 90%, the intensity of the dose was 12 rads/minute, and the radiation dose was 260 rads. In the second mode of irradiation, the neutron component was approximately 35%, the dose intensity was 30 rads/minute, and the dose of radiation was 560 rads.

The principal contribution to the neutron dose (approximately 80%) was from fast neutrons ($E > 0.4$ MeV). The average energy of the neutrons was approximately 1 MeV, while that of the gamma rays was 1.6 - 1.8 MeV.

The preparations were dissolved in distilled water and administered intraperitoneally in a volume based on the calculation of 0.1 ml/10 grams of weight of the animal, 15 - 20 minutes prior to exposure. The dose was calculated on the basis of the compound. The observations of the mice lasted 30 days.

The antiradiation effect of the preparations was evaluated on the basis of the clinical picture of radiation damage, mortality, and average survival time (AST) of the animals which died.

As the studies showed, with neutron irradiation ($LD_{90/30}$, n° ≈ 90% of the total dose) the primary factor in the syndrome of radiation sickness in the mice was damage to the intestine, accompanied by diarrhea with admixture of mucus and blood, anorexia, a drop in the motor activity and weight of the animal. It is evident from Table 1 that under these conditions the mortality of the mice during the first 5 days of sickness was 72.6%. The relative contribution of the "marrow" death to the total mortality of the animals was considerably lower (approximately 18%). This was also reflected in the low AST of the control mice - 4.4 days.

The prophylactic use of radioprotectors (AET and tsistafos) slightly decreased the severity of irradiation damage. Radioprotectors protected more than half of the mice against "intestinal" death, but by the 30th day of the sickness only 24.2% remained alive for AET, and 26.8% for tsistafos.

It is important to note that cystamine and 5-methoxytryptamine did not produce any significant radioprotective effect under these conditions. From the data in Table 1, it is also evident that joint application of AET and tsistafos with 5-MOT did not increase the antiradiation effectiveness of the protectors.

Table 2 shows data on the radioprotective properties of pharmacochemical substances under conditions of mixed gamma-neutron irradiation with a decrease in the neutron component to 35% of the total dose. In this series of experiments, the mice were subjected to superlethal doses of radiation (560 rads). Under these conditions, during the 5 days following irradiation 95% of the mice died. The AST of the control animals was 3.9 days.

TABLE 1

ANTIRADIATION EFFECTIVENESS OF PHARMACOCHEMICAL SUBSTANCES WITH MIXED GAMMA-NEUTRON IRRADIATION, WITH NEUTRON COMPONENT EQUAL TO APPROXIMATELY 90% (RADIATION DOSE EQUALS 260 - 290 RADS)

Groups	Dose, mg/kg	Number of Animals	Survival After Irradiation, %		
			5 days	30 days	AST, Days
Control	0	95	27.4±4.4	9.5±3.0	4.4±0.1
AET	150	66	56.0±6.1*	24.2±5.3*	5.4±0.2*
Tsistafos	350	56	55.4±6.7*	26.8±5.9*	5.7±0.4*
Cystamine	150	59	49.2±6.5*	5.0±2.8	4.8±0.2
5-MOT	75	68	39.8±5.9	6.0±2.9	4.6±0.2
AET+5-MOT	150+ +5	20	35.0±10.6	15.0±8.0	5.0±0.3
Tsistafos +5-MOT	350+ +75	20	60.0±10.9*	30.0±10.2	7.0±1.2*

* $p < 0.05$

The use of the investigated substances (AET, cystamine, tsistafos) showed a pronounced radioprotective effect. The preparations protected 70 - 80% of the mice against intestinal death ($p > 0.05$). By the 30^{th} day following exposure, AET, cystamine and tsistafos had produced survival of 42, 33.3 and 40% of the mice, respectively, with 100% mortality in the control group. Mexamine proved to be ineffective under these conditions.

Figures 1 and 2 show the dynamics of the weight change of the /134 control and experimental animals which were subjected to mixed

TABLE 2

ANTIRADIATION EFFECTIVENESS OF PHARMACOCHEMICAL SUBSTANCES WITH MIXED GAMMA-NEUTRON IRRADIATION, WITH NEUTRON COMPONENT EQUAL TO ABOUT 35% (RADIATION DOSE = 560 RADS)

Groups	Dose, mg/kg	Number of animals	Survival After irradiation, %		AST, days
			5 Days	30 Days	
Irradiation without protection	0	40	5.0±3.4	0	3.9±0.2
AET	150	31	67.7±8.7*	42.0±8.9	5.5±0.7*
Cystamine	150	18	83.3±8.8*	33.3±11.1	5.7±0.5*
Tsistafos	350	25	80.0±8.0*	40.0±9.8	6.2±0.7*
5-MOT	75	20	15.0±8.0	0	4.4±0.2
Control		20	100	100	—

$p < 0.05$

gamma-neutron irradiation with a neutron component of 90 and 35% of the total dose, respectively. The data indicate that the depth of the initial drop in weight of the animals was evidenced to approximately the same degree in the experimental and control mice. We did not observe any differences in the loss of weight during the initial period with mixed irradiation with neutron components of 90 and 35%.

The recovery of weight in the experimental animals was noticed beginning with the 15^{th} through 25^{th} days following exposure. In a group of experimental animals which were exposed to a gamma-neutron flux with a 90% contribution of neutrons to

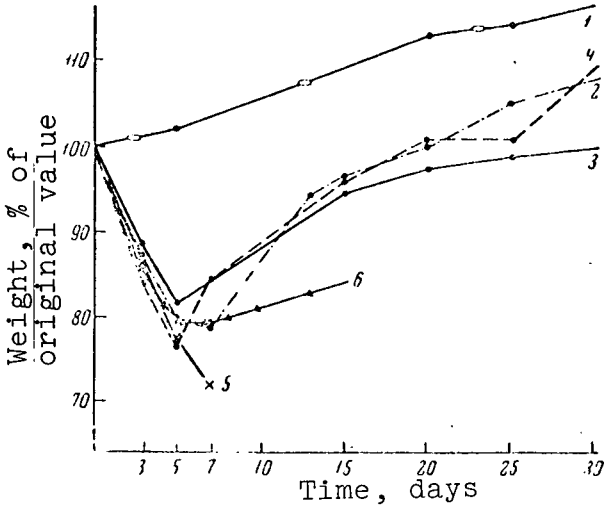

Figure 1. Dynamics of the weight of mice following mixed gamma-neutron irradiation with neutron component equal to about 90% in the total dose. 1 - control without irradiation; 2 - AET, 150 mg/kg; 3 - tsistafos, 350 mg/kg; 4 - cystamine, 150 mg/kg; 5 - 5-MOT, 75 mg/kg; 6 - control with irradiation.

the total dose, we noticed a much slower course of recovery processes than in those mice which were irradiated with mixed irradiation having a large contribution of gamma rays.

An analysis of the data in the literature [4] and our own results indicates that the effectiveness of radio-protectors from the aminothiol group with gamma-neutron irradiation depends on the ratio between these two components in the total dose of radiation. With mixed irradiation having a 90% neutron component, we did not obtain the same high degree of protection which is observed when preparations are used under conditions of x-ray and gamma irradiation or the influence of high-energy protons [5, 6]. With a 35% contribution of the neutron component, the protective effect of the aminothiol radioprotectors develops to a much higher degree. The radioprotector 5-MOT from the indolylalkyl-amine group was found to be ineffective in both varieties of mixed irradiation.

At the present time, the intimate aspects of the mechanism of radioprotective action of pharmacochemical substances have

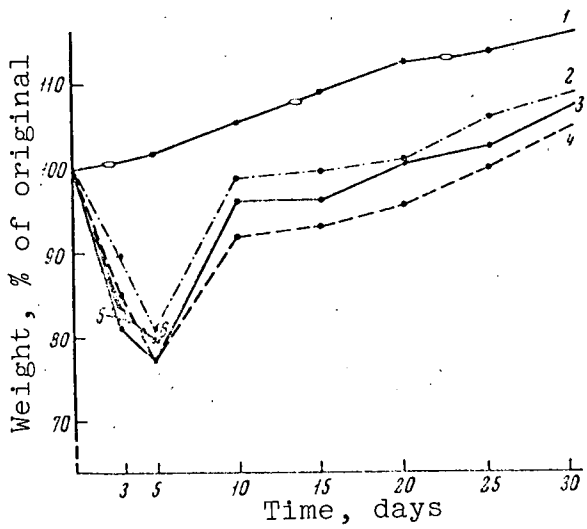

Figure 2. Dynamics of the weight of mice following mixed gamma-neutron irradiation with neutron component approximately equal to about 35% in the total dose. Symbols the same as in Figure 1.

been insufficiently investigated [1], which complicates the analysis of the protective effect of antiradiation compounds under conditions of neutron irradiation. It is possible that the poor protective properties of chemical substances under these conditions are caused by the high linear energy loss (LEL) of the radiation. Thus, we know that the protective effectiveness of indolylalkylamines to a certain extent is due to their hypoxic effect [2, 3, 12], while for the development of a biological effect of radiation with high ionization density (neutrons, alpha-particles) the role of the "oxygen" effect is negligibly small or absent.

Nevertheless, the effect which was observed, in all probability, is more complex. It is necessary to take into account the characteristics of the clinical course of radiation damage in irradiation by neutrons, in particular, the high degree of sensitivity to damage involving the intestine. Thus, the absence of a mexamine effect may have something to do with the low level of protection of the intestine tissues by indolylalkylamines [11]. The different tropicity and distribution of aminothiols in tissues (and possibly in the microstructures of the cell as well) apparently causes the lower effectiveness of

cystamine in comparison with AET and tsistafos under the irradiation conditions which we employed.

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[N73-20069]

Elimination of Cystamine in the Organism and the Prolongation of its Radioprotective Effect

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ABSTRACT. A method is proposed for determining the elimination of the toxic effect of cystamine. In the first approximation, the rate of elimination of this radioprotector in the first three hours amounts to about 25 to 35% per hour. An attempt was made to prolong the radioprotective effect by adding the eliminated dose of cystamine. It was thus possible to maintain the radioprotective level of the first dose up to two hours. Elimination of cystamine through additional multiple administration of small doses with an interval of one hour proceeds with a drop in its rate with each subsequent administration; beginning with the third administration, there is accumulation of the toxic effects of the cystamine. The decrease in the radioprotective effect of the cystamine proceeds more rapidly than its toxic effect is eliminated. Preliminary experiments have shown that this method may be used to determine the elimination of other radioprotectors as well (AET, tsistafos and 5-methoxytryptamine).

Several methods are used to detect mercaptoamines and /13
particularly cystamine in the tissues and substances of organisms: the use of sulfur- or hydrogen-labelled radioprotectors (cystamine-S^{35}), titration of sulfhydryl or disulfide groups, electrophoretic separation with a "blank". However, as was pointed out by A. N. Mozzhukhin and F. Yu. Rachinskiy [5], "not one of the existing methods provides exhaustive quantitative data on the presence in the biosubstrate of the radioprotective compound and the products of its conversion" (p. 152).

Studying the influence of radioprotectors on the ability of animals to withstand transversely directed overloads, we attempted to link the recovery of normal reactivity of the organism to overloads with a decrease of the toxic effect of cystamine with time.

In a previous work [2] we briefly presented the results of one of our experiments. It is of considerable interest to analyze this phenomenon in greater detail and to express the elimination of cystamine (determined on the basis of a decrease in the toxic effect on a second administration) by the residual dose of cystamine. In addition, as we shall see in more detail later on, this method made it possible to prolong the radioprotective effect of the cystamine.

The behavior of radioprotectors when administered orally into the stomach, in comparison with their parenteral behavior, has many specific characteristics that deal with the process of its absorption from the gastrointestinal tract.

As was revealed by studies described in this paper, the use of this method in conjunction with others can give additional information about the behavior of radioprotectors in the organism when administered orally into the stomach.

Elimination of Cystamine Following Single Intraperitoneal Administration

Principle of the Method. The method is based on an analysis of the reaction of the animal to the secondary effect of the same factor whose influence is being studied, to determine whether or not its biological effect is eliminated. By this term, we mean the attenuation of the effect of some factor with time — or, in otherwords, the recovery of normal reactivity with respect to the action of that factor.

As an example of a particular case of the general theory of recovery, we can use the theory of Blair [15] regarding the evaluation of recovery following radiation damage. /138

We can assume that any recovery of reactivity of the organism, determined quantitatively, will show a sinusoidal relationship between time and the residual effect. Recovery in this case takes place in three phases: the first is one of slow recovery, the second is one of rapid recovery, and the third is one of slow recovery once again. Depending on the nature and the properties of the active factor, recovery or reactivity will proceed with lengthening either of the first or second phase of recovery. In particular, recovery from radiation damage involves lengthening of the second phase, which agrees with the theoretical $e^{-\beta t}$ curve. Recovery of reactivity under the influence of pharmacological preparations which do not have pronounced organotropicity and possess a general cytotoxic effect, in our opinion, may have the same sinusoidal character and may be evaluated by the method of repeated testing. Cystamine possesses such properties to a certain degree.

Experimental Model of Eliminating the Toxic Effect of Cystamine. Determination of recovery from the toxic action of cystamine may be performed either on the basis of the reduction of the LD_{50} of the toxic effect of cystamine, following preliminary administration of a dose approximately equal to 1/2 LD_{50}, or by determining the dose on the basis of mortality. In the latter case, it is necessary to have a graduated curve showing the dependence of the effect on the dose. In Table 1, we have shown the results of one of the experiments in evaluating the elimination of cystamine in the organism of a mouse. Cystamine dichlorhydrate was administered intraperitoneally at a dose of /139

approximately 1/2 LD_{50}, i.e., 125 mg/kg (first dose) and 225 mg/kg (second dose). The dose was administered from calculation on the base.

The intervals between the first and second doses were 5, 20, 40, 60 90, 120, 180 minutes and one day. The zero interval corresponded to the simultaneous administration of the first and second doses. Following the second dose, we recorded the total mortality of the animals and the times of their deaths. To convert the percentage of mortality to the corresponding dose of the preparation (6th column, Table 1), we determined the dose curve for cystamine in an experiment with 140 mice.

In the following we present the analytical expression of this graduated curve:

$$P = 12{,}2 \log D - 24{,}6, \qquad (1)$$

where P — is the mortality (in victims); D — is the dose of cystamine (in mg/kg).

If recovery of reactivity were absent following the first dose of the preparation, mortality with an increase in intervals between doses would have remained unchanged. However, as is evident from the table, the mortality decreased although the total dose remained constant (350 mg/kg). Table 1 gives the effective cumulative doses (D_e) calculated by Formula (1) and the difference between D'_2 and the second dose (D'_2).*

The effective residual dose is expressed in percentage of D_1. The value of the residual dose of cystamine is decreased with an increase in the time interval (T) following administration

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Editor's Note: This must be a misprint in the original text.

TABLE 1. ELIMINATION OF CYSTAMINE IN THE ORGANISM (BASED ON THE DECREASE OF THE TOXIC EFFECT FROM THE SECOND ADMINISTRATION OF THE PREPARATION)*

Interval, minutes	Dose, mg/kg		No. of mice	Mortality, %	D_e, mg/kg	Residual dose ($D_e - D'_2$)		Average survival time of mice which died	
	D_1	D_2				mg/kg	%	M ± m min.	% of "0" interval
0	125	225	30	93,3	354	124	100	51±4,9	100
5	125	225	20	90,0	345	115	93	44±5,6	86
20	125	225	30	83,3	320	90	73	53±3,6	103
40	125	225	30	86,7	330	100	81	65±7,1	126
60	125	225	30	86,7	330	100	81	74±7,7	144
90	125	225	30	73,3	302	72	58	80±10,0	157
120	125	225	30	53,0	270	40	32	100±13,5	196
180	125	225	30	20,0	224	—6	—5	130±30,0	252
1440	125	225	20	10,0	209	—21	—17	55	107
1 day									
—	0	225	40	22,0	230 (D_2')	—	—	70±10,3	136

*Commas represent decimal points.

of the preparation. This correlation may be approximated by a victim-linear relationship, where the residual dose (%) is expressed in victims (Figure 1):

$$D_e = 6{,}58 - 0{,}0166 \cdot T, \tag{2}$$

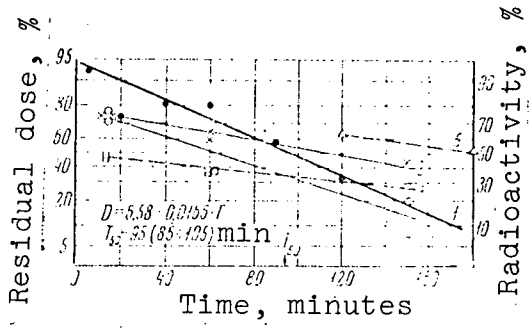

Figure 1. Behavior of cystamine in the organism of a mouse when administered intraperitoneally:

1 — elimination of cystamine based on the decrease in the toxic effect (D — dose in victims). Following administration of 150 mg/kg of cysteamine — S^{35} [17]; 2 — total radioactivity in %; 3 — the same for liver, brain and kidneys; 4 — radioactivity of S^{35} — cysteamine — cystamine; 5 — quantity of non-protein HS-groups in the liver, brain, and kidneys in rats following administration of cystamine at a dose of 100 mg/kg, in percent in a 30-min. interval (based on data in [8, 9])

where D_e is the percentage of the residual dose (in victim units), T is the time following administration of the preparation (in minutes).

By using Equation (2), we can determine the time to eliminate T of cystamine from the organism for various time intervals.

In the following, we have calculated the time of elimination (with reliable limits) of cystamine for various levels of the residual dose: the time of 50% elimination ($T_{0.5}$) of cystamine,

on the basis of the data from these experiments, was 95 (86 - 105) minutes:

T_y	Confidence limits, T_y, minutes	T_y	Confidence limits, T_y, minutes
$T_{0.8}$	44 (40 - 49)*	$T_{0.5}$	95 (86 - 105)
$T_{0.7}$	64 (57 - 63)	$T_{0.4}$	110 (101 - 122)
$T_{0.6}$	74 (67 - 82)	$T_{0.3}$	127 (114 - 141)
		$T_{0.2}$	146 (132 - 162)

*$T_y/f_t - T_y/f_T$ (p - 0.05), where y is the residual dose of cystamine, f_t = 1.11.

An analysis of the elimination of cystamine in the organism will show that during the first hour 28% (24 - 32%) of the radioprotector is eliminated, while after 2 hours 66% (59 - 74%) is eliminated. After 3 hours, the cystamine is practically completely eliminated; according to Equation (2), only approximately 7% (−3 - 17%) cystamine remains in the organism.

This agrees with the data of Verly [17] from a determination in mice (C 57 bl strain) of S^{35}-cysteamine-cystamine on the basis of the residual total radioactivity following intraperitoneal administration in 150 mg/kg of cystamine.

After 2.5 hours have passed, only 20% of the activity remains in the organism from S^{35}-cysteamine-cystamine. In Figure 1, curves 2 (total amount of S^{35}) and 4 (amount of S^{35}-cysteamine-cystamine) were plotted on the basis of the data of Verly et al [17]. On the basis of the slope of the curves, the maximum

correlation is found between the elimination of the toxic effect of cystamine and the quantity of S^{35}-cysteamine-cystamine. However, the time for half-excretion of S^{35}-cysteamine-cystamine on the basis of curve 4 (see Figure 1) is approximately 2 times less than the elimination of cystamine, while the $T_{0.5}$ for the total amount of S^{35}, on the other hand, is more than 15 minutes. According to the data of Verly et al [17], following intraperitoneal administration of 150 mg/kg of S^{35}-cysteamine-cystamine, the maximum activity was found in the liver, kidneys, spleen and pancreas. Obviously, the toxic effect of cystamine is produced by damage to such organs as the liver, brain and kidneys. We calculated on the basis of the data of Verly [17] the decrease in the residual total radioactivity of S^{35} for these organs. The total radioactivity S^{35} for these organs (Curve 3) naturally is lower in level, but its slope does not differ from Curve 2. Within 15 minutes to 6 hours, the radioactivity of the S^{35} of the liver, brain and kidneys amounted to 60 - 70% of the total activity.

Curve 5 (see Figure 1) reflects the content of non-protein /141
HS-groups (percentage at the 30-minute interval) in the liver, brain and kidneys in the rat following intraperitoneal administration of cystamine in doses of 100 mg/kg according to the data of A. V. Titov [8, 9]. The metabolites of cystamine obviously do not determine elimination of the toxic effect, inasmuch as they (sulfonocystamine, N-, S-acetylcystamine, taurine, gynotaurine, thiotaurine) are not very toxic when administered intraperitoneally [7]. It is possible that the elimination of cystamine will correspond more (but not completely) to the decrease in these organs of the amount of S^{35}-cysteamine-cystamine, but unfortunately we do not have these data.

The slight increase in resistance to repeated administration of cystamine with an interval of 3 hours and especially

one day that was observed evidently was caused not only by the presence of the preparation itself, but also by the reactivity of the organism which was changed under the influence of this protector. The following interpretation may be given to this phenomenon.

We know that continuously acting processes in biological systems function according to a feedback principle with zero delay. This phenomenon is also observed in recovery from radiation damage in animals which have been irradiated with gamma and x-rays [1, 16] and protons [11].

Hence, the curve which we obtained for the elimination of cystamine most closely reflects the reactivity of those biological structures which accept this radioprotector. We noticed the increase in the survival time of the mice in toxic death (Figure 2). With an increase in the time interval between the two administrations of cystamine, the average survival time becomes proportionally greater and by the third interval increases to 152% in comparison to a single administration at the same dose. It is interesting that even considering the elimination of the first dose (3 hours later, on the basis of the mortality test, it is completely eliminated) the survival time for the animals which die from the second dose (225 mg/kg) is still greater by 112% than is the case for a single administration of cystamine.

The average survival time of the animals which die from the second administration of cystamine with an interval of 1 day differs insignificantly from the single administration of the same dose. The increase in the time of death from the second administration of the preparation points to possible blockage of the cellular structures by the metabolites of cystamine. Therefore, the second dose has a slower toxic effect. We can assume that

in this case the protective effect of the cystamine following preliminary administration of the preparation in the same dose will be less than when it is given once.

The increase in the average survival time of the mice which die after the second administration of cystamine may have something to do with the fact that the second dose of the radioprotector is accepted slowly (or incompletely) by the cells of the organism due to their blockage by the metabolites of the cystamine from the first dose. While this assumption is valid to some extent, obviously the protective effect of cystamine from second administration will be less than from the first. We selected a three-hour interval. In this case there was no toxic death from the second administration of cystamine, although the changed functional state of the biosubstrates from the first administration of the radioprotector is apparently still retained. At later periods (after 3 hours), the negative influence of the first dose of cystamine on the radioprotective effectiveness of the second dose must unquestionably disappear.

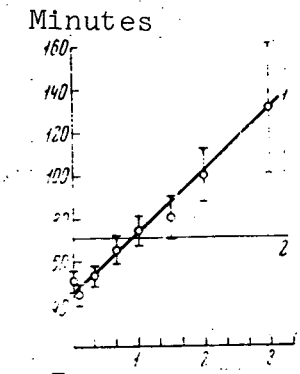

Figure 2. Average survival time (in minutes) of mice which died from toxic effects with double administration of cystamine in doses of 125 and 225 mg/kg (1) and a single administration at a dose of 225 mg/kg (2)

In Table 2, we have described a number of experiments on the radioprotective effectiveness of cystamine in two applications.

TABLE 2. INFLUENCE OF REPEATED INTRAPERITONEAL ADMINISTRATION OF CYSTAMINE (C) IN A DOSE OF 150 + 150 mg/kg ON ITS RADIOPROTECTIVE ACTIVITY IN MICE (FEMALE)

Date in 1969	Group	Experimental conditions	No. of Animals	Survival on the 30th day		AST, Days
				Abs.	%	
Feb. 11	1	C + C (3-hour interval) + 850 r	20	15	75	19.4±2.6
	2	C + 850 r	20	20	100	–
	3	850	20	0	0	9.5±2.1
	4	C + C (interval of 3 hours)	20	20	100	–
April 22	1	C + C (3-hour interval) + 850 r	20	5	25	3.7±1.5
	2	C + 850 r	20	18	90	9.0±1.0
	3	850 r	20	0	0	10.0±0.9
May 19	1	C + C (3-hour interval) + 900 r	20	2	10	2.0±0.6
	2	C + 900 r	20	12	60	16.0±2.9
	3	900 r	20	0	0	4.7±0.9
	4	C + C (3-hour interval)	10	10	100	–
	5	C + C (3-hour interval)	10	10	100	–
	6	C	10	10	100	–
	7	Control	10	10	100	–
Total data	1	C + C (3-hour interval) + irradiation	60	22	37±6.1	5.0±0.5
	2	C + irradiation	60	50	83±4.8	14.6±0.9
	3	Irradiation	60	0	0	8.1±0.7
	4	C + C (3-hour interval)	30	30	100	–

Note. In Group 1, the interval between the second administration of cystamine and the irradiation was 10 minutes. In Groups 5 - 7, the animals were placed in irradiation chambers for 25 minutes.

At a dose of 150 mg/kg, the preparation was given intraperitoneally with an interval of three hours. Ten minutes following the second administration, the animals were irradiated with 850 - 900 r. The double administration of cystamine, with an interval of 3 hours, did not produce death of the animals. Keeping such animals in irradiation cells for 25 minutes (the time required for exposure) also did not lead to death. All of the animals which were irradiated with 850 - 900 r died in the course of 8.1 $\pm$ 0.7 days. With a single administration of cystamine, 83% remained alive on the 30th day of observation (AST = 14.6 $\pm$ 0.9 days). However, a second administration of radioprotector 3 hours following the first dose led to a drop in the radioprotective effectiveness: the survival was 37% ($p < 0.001$) and the AST was 5.0 $\pm$ 0.5 days. The more rapid death of the animals (as indicated by the low AST) apparently indicates not only a drop in the radioprotective effectiveness, but also an increase in the toxicity of the cystamine due to a delay in its elimination in the organism. Preliminary experiments performed jointly with M. V. Vasin support this assumption. As a matter of fact, in those irradiated animals which were protected by cystamine, the sensitivity of the organism increases to the second administration of the radioprotector, which was not observed in the unprotected mice, i.e., the elimination of cystamine in the irradiated animals proceeds more slowly than in the healthy ones[1].

After 1 day, elimination of the toxic effect apparently is /143
complete, although the residual physiological effect is still retained. Multiple daily application of cystamine at a dose of 150 mg/kg for 12 days has no influence on its radioprotective properties (Table 3). The last dose was given 15 minutes prior to irradiation.

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[1] See the article by M. V. Vasin et al., in this collection page 256.

TABLE 3. INFLUENCE OF TWELVE-FOLD DAILY INTRAPERITONEAL ADMINISTRATION OF CYSTAMINE AT A DOSE OF 150 mg/kg ON ITS RADIOPROTECTIVE ACTION

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Experimental conditions*	Survival on the 30th day		AST, days
	Absolute	%	
150 mg/kg (12 times) + 150 mg/kg + 850 r	20	100	–
150 mg/kg + 850 r	17	85	15.5
850 r	0	0	9 ± 0.7

Note. In the control prior to irradiation, 0.2 milliliters of physiological solution were given daily for 12 days.

*There were 20 animals in each group.

Prolongation of the Radioprotective Effect of Cystamine by Additional Administration of the Eliminated Dose

We determined above that the elimination of cystamine takes place in inverse proportion to the sample of the residual dose.

In order to maintain the original level of the radioprotector in the organism for a period of several hours, it is obviously necessary to add a quantity of cystamine which is equal to 28% of the initial dose. We can then expect that the radioprotective effect of the cystamine will be retained at the original level.

In analyzing the elimination of cystamine, we did not take into account the non-eliminated toxic effect. The experiments

showed that daily introduction of cystamine at a dose of 150 mg/kg for 11 days will not lead to death of the animals. This agrees with the data in the literature [4]. If we assume that the dose of 160 - 170 mg/kg is the minimum toxic dose, the cumulative dose by the 11th day will be $\leq$ 10 - 20 mg/kg, while the level of the non-eliminated part of the cystamine in one dose will will be $\leq$ 1 - 2 mg/kg, or $\leq$ 0.7 - 1.4%.

One hour following administration of cystamine at a dose of 150 mg/kg, 42 (36 - 46) mg/kg is eliminated. The experimental test showed that, when it is administered 1 hour following a dose of 150 mg/kg, it can produce a single death (Table 4, Series 1). Hence, the calculated dose was found to be 17 (2 - 24) % higher than the dose obtained in the experiment. One and a half to two hours later, about 50 - 60% is eliminated, i.e., at a dose of 150 mg/kg — 75 - 90 mg/kg.

In order to eliminate a possible toxic effect in experiments designed for protection, we used slightly smaller doses for the second administration of cystamine (20 - 30 and 75 mg/kg) than was indicated by the experiments on elimination. The arrangement of the experiments was as follows. The mice were /145
given cystamine dichlorhydrate intraperitoneally at a dose of 150 mg/kg (calculated for the base). Then the preparation was given every hour in doses of 20, 30 and 75 mg/kg (other groups in this experiment received intraperitoneally 0.2 mm of physiological solution). After 10 minutes following the last administration, the animals were subjected to gamma irradiation at a dose of 850 r.

Tables 5 and 6 show the results of experiments on a double additional administration of cystamine at doses of 20, 30 and 75 mg/kg.

TABLE 4. TOXIC EFFECT OF CYSTAMINE AT A DOSE OF 150 mg/kg WITH ADDITIONAL ADMINISTRATION OF SMALL DOSES

Series*	Dose of Cystamine,** mg/kg				Mortality	
	1st	2nd	3rd	4th	Absolute	%
I	150	15	—	—	0	0
	150	25	—	—	0	0
	150	35	—	—	0	0
	150	45	—	—	3	15
II	150	35	5	—	0	0
	150	35	10	—	1	5
	150	35	15	—	3	15
III	150	35	7.5	5	2	10
	150	35	7.5	10	4	20
	150	35	7.5	15	6	30

*Twenty animals in each version of the experiment.

**Intervals of 1 hour between administrations of cystamine.

In addition, as a control, we set up the following variations: (1) the animals were irradiated with a dose of 850 r after 10 minutes, and 2 hours following administration of cystamine at a dose of 150 mg/kg; (2) the mice were irradiated with a dose of 900 r[2] 10 minutes following doses of 25, 50 and 75 mg/kg (data of M. V. Vasin).

[2]The survival rate and the average survival time were close to the dose of 850 r.

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TABLE 5. INFLUENCE OF MULTIPLE INTRAPERITONEAL ADMINISTRATION OF CYSTAMINE IN SMALL DOSES ON THE PROLONGATION OF THE RADIOPROTECTIVE EFFECT OF THE FIRST DOSE***

Interval between		Group	Dose, mg/kg		Radiation Dose, r	Number of animals	Survival*		AST**, days
D_1 and D_2, hours	D_1, D_2 and irradiation, min.		D_1	D_2			Abs.	%	
1	10	1	150	20×2	850	20	12 (2)	60±11,2	6,1±1,0
1	60	2	150	20×2	850	20	3 (2)	15±8,2	15,8±1,4
1	10	3	150	30×2	850	20	13 (1)	65±11,0	14,0±1,0
2	10	4	150	75×2	850	80	40 (14)	50±5,5	12,9±1,4
2	10	5	150	75×3	850	60	12 (30)	20±5,2	12,1±1,2
—	10	6	25	0	900	79	0	0	6,8±0,3
—	10	7	50	0	900	60	2	3,3±2,8	11,0±0,4
—	10	8	75	0	900	60	21	35,0±6,2	11,7±1,0
—	120	9	150	0	850	20	1	5±5,0	12,6±1,2
—	10	10	150	0	850	60	44 (2)	73±5,5	11,6±2,1
—	10	11	150	0	900	40	37	92,5±4,3	13,3±1,1
—	—	12	0	0	850	60	0	0	7,5±0,4
—	—	13	0	0	900	116	1	0,86±0,8	6,6±0,3
—	—	14 Control	0	0	0	20	20	100	—

*With consideration of toxic death. In parentheses: death from toxicity.

**Without toxic death.

***Commas represent decimal points.

TABLE 6. DEATH OF IRRADIATED MICE (850 r) FOLLOWING SINGLE (150 mg/kg) AND REPEATED ADMINISTRATION OF CYSTAMINE IN SMALL DOSES (20 - 30 mg/kg)*

Group	Dose, mg/kg		No. of mice	Days													
				0—2		3—5		6—10		11—15		16—20		21—25		26—30	
	D_1	D_2		abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
1	150	30×2	20	1	5	0	0	0	0	3	15	3	15	0	0	0	0
2	150	20×2	20	2	10	3	15	2	10	1	5	0	0	0	0	0	0
3	150	20×2	20	2	10	0	0	2	10	7	35	4	20	1	5	1	5
4	150	75×2	80	14	17,5	5	5,8	6	7,5	9	11,2	3	3,7	2	2,5	6	1,2
5	150	75×3	60	30	50	2	3,3	6	10	5	8,3	2	3,3	2	1,6	1	1,6
6	150	0	20	0	0	0	0	8	40	5	25	6	0	0	0	0	0
7	150	0	60	2	3,3	4	6,6	2	3,3	3	5	3	5	0	0	2	3,3
10	0	0	60	1	1,6	18	30	28	47	12	20	0	0	0	0	0	0

Note. For the characteristics of the groups, see Table 5.

*Commas represent decimal points.

As we can see from Table 5, the survival rate of the animals with an additional double administration of cystamine at a dose of 20 - 30 mg/kg was 60 - 65%; when the animals were irradiated 2 hours following the administration of cystamine in a dose of 150 mg/kg, it was only 5%. By themselves, doses of 25 and 50 mg/kg will not have any protective effect. With the additional administration of a dose of 75 mg/kg with an interval of 2 hours and with irradiation for 10 minutes following the 3rd and 4th doses, the survival rate was 50 and 20% respectively. In these groups, we observed a rather high mortality — 17.5 and 50%, /14 respectively, during the first two days following exposure (see Table 6). Obviously, this mortality is due to an increase in the toxic effect of the radiation. When only a dose of 75 mg/kg is given, this survival rate was only 15% less than for a multiple application of cystamine.

In Figure 3 and Table 6, we have shown the data on the dynamics of mortality and the weight of the mice for 30 days following exposure. The change in weight of the animals which were irradiated 10 minutes following administration of 150 mg/kg and 2 hours later, but with a double additional administration every hour of 30 mg/kg of cystamine per dose, was almost the same. There was an initial decrease in weight by 20%, then a gradual increase to the original level by the 20th day. The weight gain amounted to 11 - 12% and was 30% less than in the controls (Figure 3, B).

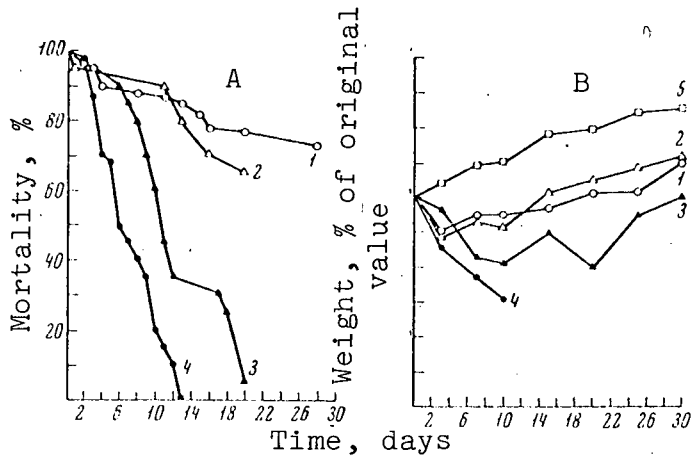

Figure 3. Influence of repeated application of cystamine in small doses on the prolongation of its radioprotective action:

A — dynamics of mortality; B — change in weight (value of scale divisions — 20%); 1 — cystamine 150 mg/kg + 850 r; 2 — cystamine, 150 mg/kg + 30 mg/kg (twice) with a 1-hour interval + 850 r; 3 — cystamine, 150 mg/kg, after 2 hours of irradiation at a dose of 850 r; 4 — 850 r; 5 — control

Having obtained a positive result in prolonging the radioprotective effect of cystamine up to 2 hours, we performed a

series of experiments at 4 and 6 hour intervals. The results of these experiments are shown in Table 7. A four-fold hourly administration of cystamine in doses of 20 and 30 mg/kg led to a significant decrease in the radioprotective effect. The survival

TABLE 7. INFLUENCE OF MULTIPLE INTRAPERITONEAL ADMINISTRATION OF CYSTAMINE IN SMALL DOSES (20 - 30 mg/kg) ON THE PROLONGATION OF THE RADIOPROTECTIVE ACTION OF THE FIRST DOSE (150 mg/kg)**

Dose, mg/kg*		Dose of radiation	No. of mice	Survival		AST, days
D_1	D_2			Abs.	%	
150	30×4	850	20	5	25±9,9	11,5
150	30×6	850	20	3	15±8,1	4,1
150	20×4	850	40	6	15±5,5	8,5
150	20×4	850	40	5	12,5±5,0	6,4
150	0	850	60	44	73±5,5	11,6
0	0	850	60	0	0	7,5

*Intervals between administrations D_1 - D_2, D_2 - D_2 — 1 hour; last dose D_2 and irradiation — 10 minutes.

**Commas represent decimal points.

of the animals was a total of 25 - 15% higher than without protection. With a six-fold administration of cystamine at a dose of 20 - 30 mg/kg every hour, after a dose of 150 mg/kg, the survival rate decreased to 12 - 15%. The decrease in the protective effect with a four or six-fold administration of cystamine at doses of 20 and 30 mg/kg apparently is related to remote toxic death from cystamine.

TABLE 8. TOXICITY OF CYSTAMINE WHEN ADMINISTERED IN MULTIPLE DOSES***

D_1	D_2*, mg/kg and No. of administrations	$(D_1 + D_2)$, mg/kg	Interval (t) between 1st & last administrations, hrs	Number of animals	Mortality in 2 days		D_e,** mg/kg	$D_e - (D_1 + D_2)$, mg/kg (Δ)	Δ/t (excreted per hour)
					Absolute	%			
150	20×2	190	2	40	4	10	190	0	0,0
150	20×4	230	4	40	5	12,5	195	— 35	— 8,7
150	20×6	270	6	60	18	30	219	— 51	— 8,5
150	30×2	210	2	20	1	5	174	— 36	—18,0
150	30×4	270	4	20	4	20	204	— 66	—16,5
150	30×6	330	6	40	17	43	230	—100	—16,6
150	75×2	300	4	80	14	17,5	202	— 98	—24,5
150	75×3	375	6	60	30	50	245	—130	—21,7
150	0	150	—	10	0	0	—	—	—

*At doses of 20 and 30 mg/kg, the intervals between administrations were 1 hour, while at a dose of 75 mg/kg they were 2 hours.

**D_e was determined on the basis of the formula $P = 11.9 \cdot \log D - 23.4$, where P is the mortality in victims, D is the dose (in mg/kg).

***Commas represent decimal points.

Table 8 shows an estimate of the toxic effect of cystamine when administered in multiple doses. With this cystamine administration method, the mortality from toxicity was observed during approximately 2 - 3 days, in contrast to the death from a single administration, when the average survival time of the mice which died was usually about 1 - 2 hours.

In evaluating this fact, P. P. Saksonov expressed the idea /14
that the remote toxic death with additional administration of cystamine at small doses may be caused by uremia or damage to the liver (of the hepatic coma type).

With a 2, 4 and 6-fold administration of cystamine at a dose of 20 mg/kg, death from toxicity of the preparation amounted to 10, 12 and 30%, respectively; at a dose of 30 mg/kg — 5, 20 and 43%; and 75 mg/kg — 17.5 and 50%.

The conversion of the percentages of mortality to the corresponding effective doses of cystamine will show that, between the cumulative doses of cystamine and the corresponding effective doses, there is a deficit which reflects the magnitude of the eliminated dose (see Table 8). For a 2-hour interval it amounted to 0 - 36 mg/kg; for a 4- and 6-hour interval, it was 35 to 66 and 51 - 100 mg/kg, respectively. On conversion to 1 hour, the average eliminated dose of cystamine for a double administration was 9.0 and 12.5 mg/kg per hour for a four- or six-fold administration. The somewhat higher value of eliminated dose for 1 hour was obtained when 75 mg/kg were given — 21.7 - 24.5 mg. It should be pointed out, however, that in these groups the intervals between administrations were 2 hours. Therefore, different elimination characteristics are possible.

On the average, for doses of 20 and 30 mg/kg, this value will be approximately 10 mg/kg, or about 7% of the administered dose, which differs markedly from the values which were obtained with a single administration of cystamine: 28% (calculated value) and 23% (experimental value).

The differences in elimination of cystamine with single and multiple administration gave us a basis for expressing a

hypothesis regarding the reduction of the elimination rate of this preparation with each successive administration.

Elimination of the Toxic Effect of Cystamine when Administered in Multiple Doses

In this experiment, between the first dose of cystamine (D_1, 140 mg/kg as a basis) and the test dose (D_{test}, 120 mg/kg) we administered intraperitoneally 20 mg/kg of the same radioprotector. The intervals between the administrations of the individual doses were 1 hour each.

We have presented below a brief description of the experimental groups and results (Table 9). We are struck first of all by the postponed death of the animals with multiple administration (Figure 4).

Table 10 shows the sequence of the calculations for determining the rate of cystamine elimination following each successive administration of an additional dose. The calculations show that the rate of elimination per hour is 35 mg/kg. This is in good agreement with the experimental values obtained previously. In addition, the additional administration of 20 mg/kg reduces the rate of elimination to 7.2 mg/kg. One is especially struck by the fact that, with a double (or higher) administration of cystamine at a dose of 20 mg/kg, there is a complete absence of its excretion and, on the other hand, there is even an accumulation in the organism.

The tests by a slightly different experimental method of a decrease in the rate of elimination showed good agreement (see Table 4, Series II and III).

TABLE 9. MORTALITY OF MICE WITH MULTIPLE ADMINISTRATIONS OF CYSTAMINE

Interval, D_1 – D_{test} hours	Diagram of experiment	Total dose, mg/kg	No. of mice	Mortality on 1st day		Mortality on 2nd day	
				abs.	%	abs.	%
1	$D_1 + D_{test}$	260	20	3	15	3	15
2	$D_1 + D_2 + D_{test}$	280	20	4	20	5	25
3	$D_1 + D_2 + D_3 + D_{test}$	300	30	6	20	6	20
4	$D_1 + D_2 + D_3 + D_4 + D_{test}$	320	15	4	27	9	60
5	$D_1 + D_2 + D_3 + D_4 + D_5 + D_{test}$	340	15	7	47	13	87

Note. D_1 — 140 mg/kg, D_{2-5} — 20 mg/kg, D_{test} — 120 mg/kg.

In Figure 5, Curve 1 shows elimination of cystamine following additional administration. At the same time, this same figure shows the survival of the animals with this administration of cystamine. It is evident from the figure that even the second administration of cystamine at a dose of 20 mg/kg will inhibit subsequent elimination. The survival during this time still remains at a high level.

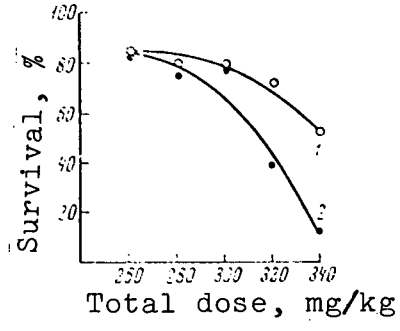

Figure 4. Survival of mice with multiple administration of cystamine with observation on the first (1) and second (2) days

Subsequent additional administration of cystamine led to a decrease in survival. Obviously, the decrease in the radioprotective effect of the cystamine with this method of administration is not /15 due solely to the mortality from toxicity (compare Tables 7 and 8).

TABLE 10. ELIMINATION OF CYSTAMINE WITH MULTIPLE ADMINISTRATION IN SMALL DOSES (20 mg/kg)

Indices	Total dose, mg/kg				
	260.0	280.0	300.0	320.0	340.0
Interval between first and test administrations of cystamine, in hours	1	2	3	4	5
Mortality, %	15	25	20	60	87
Actual dose*	224.0	240.0	230.0	290.0	325.0
Elimination, Δ mg/kg	36.0	40.0	70.0	30.0	15.0
Δ, mg/kg/hr (1)	36.0	20.0	23.0	7.5	3.0
Approximate value (1)**	35.0	21.1	13.0	7.2	2.5
Elimination of cystamine (in mg/kg) for each successive hour	−35.0	−7.2	+3.2	+10.2	+16.3

*Determined by the formula $P = 12.2 \cdot \log D - 24.6$, where P is the mortality (in victims); D is the dose of cystamine (in mg/kg).

**Determined according to the formula $D_t = 35 - 46.6 \cdot \log t$, where D_t is the dose (in mg/hg/hr); t is the time interval (in hours) between D_1 and D_{test}.

If we compare the radioprotective effects of cystamine, in the case of postponed irradiation, with the effect from the same dose which will agree with Equation (2), we will see that the radioprotective activity decreases more rapidly than the elimination. To obtain the values of the effect for any time interval between administration of cystamine and irradiation and the effect from the preparation dose, it is necessary to have the corresponding interpolation formulas. In Figure 6, we have 2 relationships: the effect of cystamine at a dose of 105 mg/kg as a function of

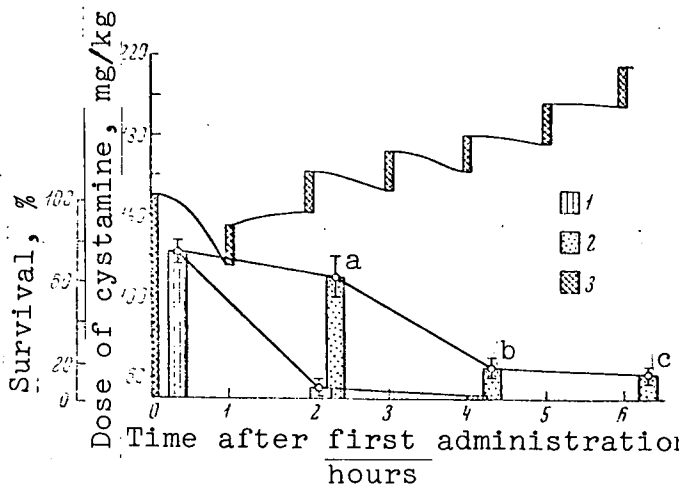

Figure 5. Radioprotective effect of cystamine with delayed irradiation and additional administration of eliminated dose: 1 — Survival with single administration of cystamine (150 mg/kg); 2 — the same, with multiple administration at intervals of 1 hour; a — 150 mg/kg + 20 mg/kg × 2; b — 150 mg/kg + 20 mg/kg × 4; c — 150 mg/kg + 20 mg/kg × 6; 3 — diagram of administration and elimination of cystamine

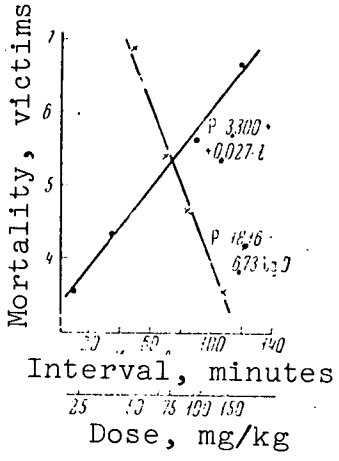

Figure 6. Dependence of the mortality of mice on the time interval between administration of cystamine (150 mg/kg) and gamma radiation at a dose of 900 r (1) and the preparation dose (2) at the same radiation dose

time interval up to irradiation at a dose of 900 r (Curve 1) and the "dose effect" (Curve 2). In Table 11 we have listed the calculations for the comparative evaluation of the survival of mice under these two experimental conditions. As these data indicate, the difference between L_D and L_t is most evident at doses of 75 - 125 mg/kg. Hence, it is obvious that the radioprotective activity of cystamine decreases more rapidly with

time, as indicated by the curve for elimination of cystamine. We know also that the degree of protection and the quantity of both free and bound protein of cysteamine-cystamine are not related in any way [14].

TABLE 11. COMPARISON OF THE RADIOPROTECTIVE EFFECT OF CYSTAMINE (D_1, 150 mg/kg) WITH DELAYED IRRADIATION AND DOSE OF CYSTAMINE CORRESPONDING TO THE CURVE OF EXCRETION (D_2 FOR 10 MINUTES PRIOR TO IRRADIATION). DOSE OF GAMMA RADIATION EQUALS 900 r (AST = 6.7 - 7.4 DAYS)*

D_2 (in mg/kg) for time t	Time (t) of elimination of D_1 up to a dose D_2, or the interval between D_1 and irradiation, min	Mortality, %		$(L_D - L_t)$ %
		At D_2 (irradiation for 10 minutes) (L_D)	At D_1 (irradiation for time t) (L_t)	
25	153	100,0	100,0	0,0
50	121	95,5	95,4	+ 0,1
75	95	69,0	84,2	—15,2
100	70	38,2	63,6	—25,4
125	38	16,2	26,6	—10,4
150	0—10	6,6	7,3	— 0,7

*Commas represent decimal points.

L_D was determined on the basis of the formula $P = 18.16 - 6.73 \cdot \log D$ and $L_t - P = 3.300 + 0.027\ t$, where D is the dose (in mg/kg), t is the time (in minutes) and P is the mortality (in victims).

Behavior of Cystamine in the Organism when Administered Orally into the Stomach

We can say a priori that the effect of cystamine when administered orally will be different than when it is administered intraperitoneally. Its effectiveness in this case will be governed by two processes: absorption from the gastrointestinal tract and elimination (metabolization and excretion). Therefore, the concept of "behavior" is more correct in this case.

The diagram of this experiment for evaluating the behavior of cystamine in the organism was theoretically the same as in the preceding experiments. At specific time intervals following oral administration (550 mg/kg for the base), we gave 150 mg/kg intraperitoneally. (These two doses, when used separately, did not produce a toxic effect). /154

The experiments were performed on 230 female mice. The results of the corresponding calculations to determine the parenteral dose of cystamine in the organism are shown in Table 12.

The effective parenteral dose of cystamine was determined on the basis of the graduated "dose-effect" equation for a given experiment:

$$P = 12.5 \cdot \log D - 25.1 \quad (n = 50 \text{ animals}).$$

We obtained good agreement with Equation (1). According to the calculations, during the first 10 minutes following the oral administration of cystamine the integral dose was 152 mg/kg, or 27.6% of the administered dose. Of course, this value in no way reflects the amount of cystamine absorbed from the stomach, inasmuch as the process of elimination also takes place

TABLE 12. ABSORPTION AND ELIMINATION OF CYSTAMINE IN MICE WITH ORAL ADMINISTRATION***

Interval (D_1 - D_2), minutes	(D_1+D_2), mg/kg	No. of animals	Mortality		D_e**, mg/kg (parenterally)	(D_e–D_2), mg/kg	In % of D_1	AST	
			abs.	%				Minimum	%
10	700	40	33	82	302	152	27,6	121±7,2	100
20—30	700	30	19	63	275	125	22,8	89±10,8	88
40	700	10	7	70	282	132	24,0	Not determined	—
60	700	30	15	50	255	105	19,0	63±6,0	52
120	700	30	18	60	269	119	21,6	78±9,0	64
180	700	10	9	90	316	166	30,0	Not determined	—
240	700	20	14	70	282	132	24,0	90±9,0	74
360	700	30	9	30	234	84	15,2	96±9,7	79
480	700	10	3	30	234	84	15.2	Not determined	—
Control with D_1	550	10	0	0	≈150	≈0	—	—	—
Control with D_2	150	10	0	0	150	0	—	—	—

*Method of administration: D_1 — orally, 550 mg/kg; D_2 — intraperitoneally, 150 mg/kg.

**D_e — determined by the expression $\log D_e = (P + 25.1)/12.5$.

***Commas represent decimal points.

during the period of actual accumulation of cystamine in the organism. However, this value reliably reflects the content of cystamine in the organism at a given moment. It is interesting that the LD_{50} of cystamine in mice for intraperitoneal administration is approximately 26 - 28% of the LD_{50} for oral administration.

It is obvious that the level of the parenteral content of cystamine with oral administration will be quite constant for a specific species of animal and a specific radioprotector.

In studying the absorption of cystamine from the stomach, A. V. Titov [8-10] observed that during the first hour rats absorbed about 3.4% (6.8% in two hours when 500 mg/kg were given orally), while for guinea pigs the figure was 35% (42 - 50% two hours after oral administration, 250 mg/kg) while for dogs it was 79.3% (50 mg/kg orally). The very slow absorption of cystamine in the stomach of the rat is not completely understandable. In this case, it is very difficult to explain the radioprotective effect of cystamine when given orally [6,13]. Our data apparently are closer to the results obtained by A. B. Titov in his experiments on guinea pigs. Such a comparison, however, must be carried out with consideration of some features of absorption in guinea pigs and mice. The corresponding maximum value of the radioprotector in the organism is observed up to 10 minutes. However, the average survival time of the animals which died from the toxic effect during this period was much higher (121 $\pm$ 7.2 minutes) than in subsequent periods (89 $\pm$ 10.8 minutes), or only for intraperitoneal administration (see Table 1). It is possible that lengthening the survival time is related to continuous absorption of cystamine in the organism. Therefore, absorption obviously ends not in the 10^{th} minute, but somewhat later, in the 30^{th} to 40^{th} minutes. Three hours after intraperitoneal administration of cystamine, the toxic effect is practically

nonexistent, as indicated by this method, but after administration /155
to the stomach even after 6 - 8 hours we could detect about 15.2% of the radioprotector (or its metabolites) responsible for the toxic effect. Obviously, we can consider the amount of cystamine that remains in the gastrointestinal tract to be a tentative value.

According to the data of A. V. Titov [8 - 9], about 65 - 75% of the administered dose remains in rats after 6 - 8 hours(3). A simple calculation will show that in our experiments after 6 - 8 hours the gastrointestinal tract still contains $\lesssim$ 50% of the administered dose. Obviously, this value is less, inasmuch as the processes of absorption and elimination take place at the same time. In Figure 7, Curve 1 reflects the behavior of the cystamine absorbed from the gastrointestinal tract. In the first approximation, this curve may be approximated by a parabolic relationship. However, with a more detailed analysis of the curve, this kind of approximation is not completely valid, in our opinion. As a matter of fact, the curve has a sort of 3-phase nature. In the first phase, there is a decrease of the dose; there is accumulation in the second and again a decrease in the third with a slower rate. If we extrapolate the portion of the curve which reflects the elimination of cystamine (Curve 3) and compare it with a curve for elimination with intraperitoneal administration (Table 1, Curve 2, Figure 7), we will see a better agreement. The segment of Curve 1 reflecting the accumulation of cystamine obviously can also be extrapolated according to exponential or logarithmic laws, which quite frequently describe biological processes. In the first approximation, the behavior of cystamine in the organism is governed by three systems called the gastrointestinal tract (which we shall call S_1),

(3) The values we obtained by interpolation between the 4- and 16-hour experimental points were 79.3 and 35.8%, respectively.

the internal sphere of the organism with its regulatory mechanisms (S_2) and elimination, which we shall call S_3. D_1, D_2, and D_3 are the doses in systems S_1, S_2 and S_3, while V_1 and V_3 are the rates of absorption and elimination, respectively.

We can make a number of assumptions regarding the behavior of cystamine when it is administered orally to the organism.

Obviously, S_1 and S_3 are functionally related to S_2 and are governed by it. Hence, S_2 obviously establishes a certain relationship between D_1 and D_2. On the other hand, the presence of a parenterally determined dose of cystamine has an inhibiting effect on the absorption process in the stomach. If this were not so, the toxicity of cystamine would be the same for the different methods of administration. We decided to test experimentally to find out whether or not it was possible to have cystamine absorption blockage in the stomach in the presence of a certain parenteral dose of protector. As a matter of fact, as the experiment showed, the intraperitoneal administration of cystamine at a dose of 150 mg/kg, and the subsequent administration of 500 mg/kg into the stomach 5 minutes later doubles the average survival time of the animals which died from the toxic effect, as compared with the reverse order: 300 and 155 minutes, respectively, for intraperitoneal-oral and oral-intraperitoneal administration. The first signs of a disturbance of the animals with the first oral administration of cystamine began 40 - 45 minutes later than with the reverse order of radioprotector administration.

When cystamine is administered to the stomach, the level of D'_2 in the S_2 system is attained at a rate V'_1 (Figure 7, A),

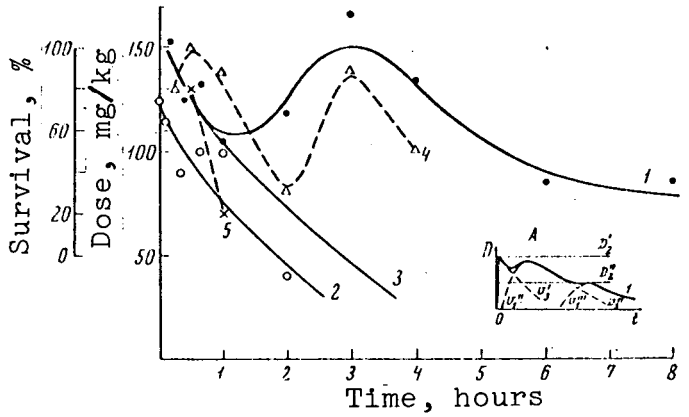

Figure 7. Behavior of cystamine in the organism with oral administration at a dose of 550 mg/kg (1):
2 — elimination of cystamine with intraperitoneal administration at a dose of 125 mg/kg; 3 — probable elimination with the assumption that cystamine is no longer absorbed from the gastrointestinal tract; 4 and 5 — survival of mice following x-radiation at a dose of 800 and 1100 r, with administration of cystamine (400 mg/kg) to the stomach [12]; A — analysis of Curve 1: v_1', v_1'', v_1'''... — rates of absorption; v_3', v_3'' ... — rates of elimination; D_2', D_2'' ... — dose levels

after which elimination begins at a rate of V'_3. When a certain level of cystamine is reached in the S_2 system, absorption again begins up to the level $D''_2 < D'_2$ at a rate of $V''_1 < V'_1$ and subsequent elimination $V''_3 < V'_3$. Most likely, absorption and elimination take place simultaneously. However, the rates of these processes, which are linked in inverse proportion through system S_2, decrease with time. The fact that the rate $V'_3 > V''_3 > V'''_3$ ... and $V'_1 > V''_1 > V'''_1$ ... is indicated by data from

experiments with fractioned administration of cystamine (see Tables 7 and 8).

The behavior of cystamine in the organism with this type of administration indicates non-linear relationships in the interaction of the organism with cystamine. Figure 7 also shows the data of Z. Bacq [12] concerning the survival of mice (C 57 bl) /157
on the 30th day with irradiation by x-rays (200 kV) at a dose of 800 r (Curve 4) and 1100 r (Curve 5) after different time intervals following oral administration of cystamine (400 mg/kg). When we compare Curves 1 and 4, we see a rather good correlation between the radioprotective activity and the level of cystamine in the organism.

In conclusion, the results of the studies which were performed may be summed up as follows:

1. A method of determining the elimination of the toxic effect of cystamine is discussed. In the first approximation, the rate of elimination of this radioprotector during the first three hours amounts to about 25 - 35% per hour. Preliminary experiments have shown that this method can determine elimination of other radioprotectors as well (AET, 5-MOT, tsistafos).

2. The elimination patterns of the radioprotector, especially cystamine, have made it possible to make an attempt to prolong the radioprotective effect of the protector by administration of the eliminated dose. Hence, it has been possible to maintain the radioprotective level of the first dose of cystamine up to 2 hours. Further prolongation with small doses (up to 4 - 6 hours) has thus far been unsuccessful.

3. The analysis performed on the elimination of cystamine with addition of multiple doses in small amounts at intervals of 1 hour showed that the rate of elimination decreases with each subsequent administration, and the dose begins to accumulate starting with the third administration.

4. The decrease in the radioprotective effect of cystamine with intraperitoneal administration proceeds more rapidly than its elimination.

5. The process of absorption and elimination of cystamine in the organism with oral administration has a sinusoidal nature, and apparently reflects both the process of absorption and the process of elimination. During the first 10 minutes following administration, approximately 27% is absorbed parenterally. However, if we take into account the duration of the toxic effect, the absorption process of the initial dose of cystamine apparently ends by the 30^{th} to 40^{th} minute.

6. Six to 8 hours later, the parenteral dose amounts to about 15% of that given to the stomach. The approximate calculation shows that in this period of time $\lesssim$ 50% of the administered dose remains in the gastrointestinal tract.

7. The behavior of cystamine in the organism with oral administration indicates non-linear relationships: the functional state of the organism, altered under the influence of cystamine, determines the rates of absorption and elimination, which decrease with time.

8. The radioprotective activity of cystamine when administered to the stomach is well-correlated with the curve of accumulation and elimination.

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N73-20070

Influence of Gamma Radiation on the Elimination of the Toxic Effect of Cystamine

M. V. Vasin, B. I. Davydov, V. V. Antipov and P. P. Saksonov.

ABSTRACT. The time of half-elimination (T_{50}) of the toxic effects of cystamine is compared in irradiated and nonirradiated mice. The authors calculated the residual dose of cystamine three hours following administration of the preparation based on the change in the protector LD_{50}. With prophylactic use of cystamine, gamma-irradiation at a dose of 850 r led to an increase in T_{50} from 1.8 (1.6 - 2.0) hours in intact mice to 2.3 (2.0 - 2.6) hours in irradiated animals. Three hours after preparation was used, the residual dose of cystamine was 44.2% in the irradiated mice and 24.5% of the original value in the nonirradiated animals. It is suggested that lengthening the elimination time of the toxic effect of cystamine under the influence of radiation is due to the decrease in the protector metabolism intensity under these conditions.

It has been shown in a number of studies [2, 5] that the sensitivity of irradiated animals varies considerably with respect to medicinal preparations made from various pharmacological groups (cardiovascular substances, narcotics, tranquilizers, analgesics, analeptics, etc.). It is definitely interesting to study the influence of ionizing radiation on the pharmacological properties of antiradiation compounds, particularly cystamine.

Data on the reactivity of the irradiated organism to these preparations are quite limited.

These problems are closely linked to the influence of radiation on the metabolism and excretion of the preparation from the organism. A. M. Rusanov et al. [4] observed an increase in the concentration of S^{35}-MEA and AET in the organisms of irradiated mice and rats by comparison with intact animals. Excretion of S^{35} in the irradiated animals was less intensive than in the non-irradiated ones. S. Ya. Arbuzov et al. [1] also observed a decrease in the rate of excretion of S^{35} -MEA under the influence of radiation, but the radioactivity of the tissues of irradiated rats was slightly less than in the non-irradiated ones. A. G. Sverdlov et al. [6] studied the metabolism products of cystamine-S^{35} in the organism of irradiated and nonirradiated mice and found a sharp decrease in the content of disulfide and thiol groups in the irradiated animals, while the quantity of oxidized forms showed almost no change. Using the method of eliminating the toxic effect of radioprotectors, especially cystamine [3], an attempt was made in this paper to study the influence of gamma radiation (during the first six hours following exposure) on the elimination of cystamine in the organism.

The experiments were performed on random-bred white female mice weighing 20 - 25 grams. The animals were subjected to gamma radiation at a dose level up to 850 r at an intensity of 34 - 35.5 r/minute. Cystamine dichlorhydrate was dissolved in distilled water and injected into the mice intraperitoneally in amounts of 0.1 ml/10 grams of animal weight. In the first series of experiments, the investigators determined the elimination time (T_{50}) of the toxic effect of the preparation using

the calibration equation

$$P = -30{,}1 + 14{,}3 \cdot \log D.$$

Cystamine was administered 10 to 15 minutes prior to exposure. The toxicity of the cystamine was measured on the basis of mortality from poisoning during the first days.

In a second series of experiments, we determined the residual dose of cystamine (150 mg/kg) three hours after administration based on the change in LD_{50}.

The results of the first series of experiments are shown in Table 1. Gamma irradiation of the mice at a dose of 850 r produced total mortality of the animals with an average survival time of 8.8 $\pm$ 0.65 days. Cystamine at a dose of 150 mg/kg used for prophylactic purposes prior to irradiation protected 84.0% of the mice for 30 days after irradiation. As we can see from Table 2, under these conditions the irradiation causes an increase in the half-elimination time of the toxic effect of cystamine up to 2.3 (2.0 - 2.6) hours. In the nonirradiated mice, T_{50} was 1.8 (1.6 - 2.0) hours. This is in good agreement with the value obtained earlier [3]. Figure 1 shows the lines of regression of the residual dose of the preparation as a function of the time following administration of the subtance to irradiated and nonirradiated mice. On the basis of the regression equations of these curves, the residual dose of cystamine 3 hours following administration of the preparation at a dose of 150 mg/kg in irradiated mice is 51 mg/kg, and 23.2 mg/kg in nonirradiated mice.

In the second series of experiments, we performed an evaluation of the calculated data obtained by changing the LD_{50}.

TABLE 1. INFLUENCE OF GAMMA RADIATION ON THE ELIMINATION OF CYSTAMINE WHEN USED FOR PROPHYLAXIS*

Dose of radiation, r	Interval, (D_1–D_2), hours	Dose of cystamine mg/kg		Number of mice	Mortality, %	D_e, mg/kg	Residual dose	
		D_1	D_2				mg/kg	%
850	1,0	135	225	20	90,0	346,7	121,7	90,0
	2,0	150	225	15	73,5	316,2	89,2	59,5
	2,5	150	225	10	40,0	275,4	50,4	33,7
	3,0	150	225	15	45,6	277,3	52,3	34,9
	4,0	150	225	15	33,3	263.0	38,0	25,3
0	1,0	135	225	20	85,0	334,2	109,2	81,5
	1,5	150	225	15	80,0	325,8	100,8	67,2
	2,0	150	225	15	53,3	288,4	63,4	42,3
	2,5	150	225	10	30,0	263,0	38,0	25,4
	3,0	150	225	15	20,0	247,2	22.2	14,8
	4,0	150	225	15	26,6	258,8	33,8	22,5
	---	0	225	15	6,7	223,9	—	—

Note. D_1 — administered 10 - 15 minutes prior to exposure, D_e determined from equation $\log D_e = (P + 30.1)/14.3$.

*Commas represent decimal points.

TABLE 2. RESIDUAL DOSES OF CYSTAMINE THREE HOURS FOLLOWING ADMINISTRATION OF THE PREPARATION (D_1, 150 mg/kg) IN IRRADIATED AND NON-IRRADIATED MICE (ON THE BASIS OF LD_{50})

Group	No. of mice	LD_{50}, mg/kg	Confidence limits at P = 0.05	Slope function	Residual dose mg/kg
D_1 + 850 r + LD_{50}	200	218.8	206.0 - 231.8	1.22	66.3
850 r + LD_{50}	134	298.5	284.0 - 313.0	1.18	-13.4
D_1 + LD_{50}	160	248.3	236.3 - 260.4	1.21	36.8
LD_{50}	125	285.1	272.8 - 298.3	1.18	0.0

Note. The interval between D_1 and irradiation is 10 to 15 minutes, and between D_1 (or irradiation) and LD_{50} — 3 hours.

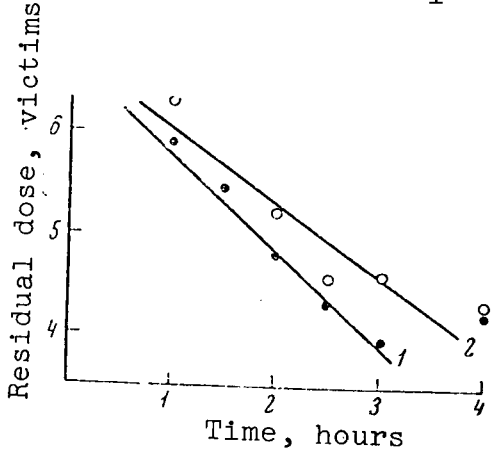

Figure 1. Elimination of cystamine without irradiation (1) and against a background of gamma irradiation at a dose of 850 r (2)

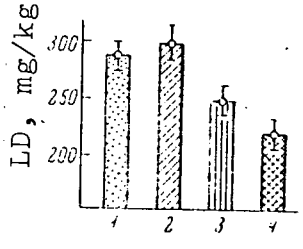

Figure 2. LD_{50} of cystamine 3 hours after its administration at a dose of 150 mg/kg without irradiation (3) and against a background of gamma irradiation (4):

1 — LD_{50} — control; 2 — LD_{50} — 3 hours after gamma irradiation

In Table 2 and Figure 2 we have listed the results for the determination of the toxicity of cystamine in intact and experimental animals 3 hours following exposure. The LD_{50} for cystamine was 285.1 (272.8 - 298.3) mg/kg. Three hours following irradiation with a dose of 850 r, the LD_{50} for the cystamine was practically the same and amounted to 298.3 (284.0 - 313.0) mg/kg. Three hours following administration of cystamine at a dose of 150 mg/kg, the LD_{50} of the preparation was 248.3 (236.3 - 260.4) mg/kg. Hence, the residual dose of cystamine for the given period of time following administration of the preparation was 36.8 mg/kg, or 24.5% of the original value. Under the conditions of irradiation 10 - 50 minutes following administration of a radioprotective dose of cystamine, the LD_{50} of the preparation after 3 hours was 218.8 (206.0 - 231.8) mg/kg. The residual dose of cystamine under these conditions was 66.3 mg/kg, i.e., 44.2% of the administered dose. These values are close to the calculated values which were obtained by using the method of the calibration curve. Consequently, gamma irradiation has a definite influence on the metabolism of cystamine used for prophylactic purposes and promotes a retention of approximately 20% of the administered dose of the substance by the third hour following administration, as determined on the basis of the toxic effect of the preparation.

On comparing the experimental data we obtained (on elimination of toxic effects of cystamine in mice) with papers of other investigators which were devoted to the study of the metabolism and excretion of the protector from the organism, certain complications arise in connection with the different conditions under which the experiments were performed: the species of animals to which the substance was given, the chemical methods and the method of expressing the results. In one of the early

experiments by Verly et al. [15], the content of S^{35}-MEA was determined in the tissues of mice after various periods following administration of the preparation, which took place 40 minutes, 1, 3 and 4 hours, with 50, 35, 15 and 10%, respectively, of the administered dose of the substance. These data were supported in the studies of Mundy [14] and Heiffer et al. [13] which deal with determining thiol groups in the blood of dogs and rats following administration of MEA in a dose of 100 mg/kg. Forty-five to 50 minutes following application of the preparation, the content of SH-groups amounts to 50% of the maximum value, and after 4 - 5 hours it returns to the original level. In determining the concentration of free and protein-bound cysteamine-cystamine following intraperitoneal administration of cystamine to rats, Betz et al. [9] found a decrease in the content of the preparation by 50% from the maximum level in the blood after 20 - 30 minutes, in the marrow after 10 minutes, the spleen after 30 - 40 minutes. When only the cysteamine-cystamine bound with protein was considered, this took place at earlier times. /162

Data on metabolism and excretion of cystamine in animals are more limited. Regardless of the fact that cystamine is restored relatively rapidly in the organism, the metabolism of cystamine (like its distribution and excretion from the organism) differs to some degree from the volume of cysteamine [10, 11]. According to the data of Mundy [14], the maximum concentration of the thiol groups in the blood of dogs following administration of cystamine (100 mg/kg) is found after 25 minutes, and drops by a factor of 2 in 70 minutes. According to the data of Gensicke et al [12], a larger portion of cystamine-S^{35} is absorbed in the tissues of mice during the 15 minutes following administration of the preparation, while the activity of the blood at the same time is 9.0%. Comparing our data with those

in the literature, it is evident that the elimination of the toxic effect of cystamine takes place somewhat more slowly than the excretion of the preparation as determined by other methods.

The modifying effect of irradiation on the metabolism of cysteamine was determined by a number of investigators [1, 4, 6, 7, 8]. Thus, Rusanov et al. [4] noticed a more intensive inclusion of MEA-S^{35} in the organs, and a more prolonged retention in those of irradiated rats than those of nonirradiated animals. To a certain degree, this fact correlates with a decrease in the elimination rate of the protector toxic effect in irradiated animals. According to the data of Sverdlov et al. [6], when cystamine-S^{35} is used for prophylactic purposes 20 minutes prior to exposure in a dose of 900 r, the total balance of S^{35} which was excreted with the urine of the irradiated mice was 30% less than for the nonirradiated animals. Obviously the toxic effect of cystamine is due mainly to the protector and its reduced form, and the marked decrease in the elimination rate of the toxic effect of cystamine in mice under the influence of radiation, in all probability, is due to a decrease in the intensity of the metabolism of the protector and not with an inhibition of metabolism product excretion.

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Influence of Shielding Certain Parts of the Body on Radiation Sickness and Survival of Animals with Total Gamma-Neutron Irradiation

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ABSTRACT. In experiments on rats subjected to total gamma neutron irradiation with a neutron component of approximately 90%, it was found that with equal masses of protected tissue the effectiveness of shielding the head is much less than the effect of shielding the anterior part of the stomach. The effect of shielding equal masses of tissue of the stomach and head under the influence of fission neutrons with an average energy of approximately 1 MeV is close to the effect of shielding these same regions of the body with irradiation with protons with an energy of 120 MeV and is considerably worse than the effect of shielding for gamma-irradiation. With a total gamma-neutron irradiation, the drop in the neutron contribution

to the absorbed dose from 90 to 35% leads to a considerable increase in the effectiveness of shielding the head and stomach. The effect observed resembled the effect of shielding for "pure" gamma radiation.

In the papers published previously [1, 2, 3], the influence of shielding individual parts of the body (head, region of the stomach) on the survival of animals and other phenomena of radiation damage with total irradiation by protons with energies of 120 MeV and gamma-rays was shown.

In this article, we present the results of studies in which the influence of shielding the above-mentioned parts of the body on the course of radiation sickness and its outcome was studied. Gamma-neutron irradiation was used, with various contributions of neutrons to the absorbed dose. An attempt was also made in this work to carry out a comparative analysis of the effect of shielding with various forms of ionizing radiation.

The experiments were performed with random-bred sexually mature rats of both sexes. Prior to the experiment, the animals were weighed and marked, while in some groups of rats we also studied the basic indices of the peripheral blood. The period of observation of the animals following irradiation was 30 days. /164
The surviving animals were weighed regularly, and the blood was tested in the rats in hematological groups on the 3, 5, 7, 10, 15 and 30th days.

The shielding effectiveness was evaluated basically by determining the survival indices for 30 days following irradiation and the average survival time (AST) of the animals which died. In addition, we determined the changes in the most

characteristic clinical symptoms of radiation sickness, especially the periods of development and the degree of severity of diarrhea, the dynamics of body weight, and in some experiments the changes in certain indices of the peripheral blood as well.

The rats were irradiated in a fixed condition in special stands. For shielding, we used 2-layer blocks, made from plastic (polymethylmethacrylate), 15 centimeters thick, and lead 1 centimeter thick, which cut down the neutron flux by 4.6 times, and the gamma radiation — 2.3 times. The blocks were mounted in front of the stands in a vertical position, with the lead layer against the body of the animal.

To shield the head, we used blocks about 10 cm wide, while for shielding the stomach the blocks were 2 and 4 cm wide. The blocks we used completely protected the head along with the anterior cervical vertebrae and the anterior extremities without the shoulder girdle. The mass of protected tissue in this case amounted to about 12 - 15% of the body weight. When shielding the portions of the stomach which were located immediately ahead of the umbilical line, the mass of protected tissue for a block 2 cm wide ("small protection of the stomach") was equal to 10 - 12%, and when protected by the 4 cm block ("large protection of the stomach") it was 20 - 25% of the mass of the body.

In each experiment, and in large experiments in each individual section, we simultaneously irradiated the protected (experimental) and unprotected (controlled) animals.

Irradiation of the animals was performed on a uranium research reactor with mixed gamma-neutron radiation. We used two modes of exposure: in the first mode, the contribution of neutrons to the absorbed dose (neutron component) amounted to

approximately 90%, while in the second mode it was about 35%. The intensity of the dose of gamma-neutron radiation in the first case was equal to 12, and in the second — approximately 30 rads/minute.

The principal contribution to the neutron dose (approximately 80%) came from fast neutrons (E > 0.4 MeV), while the thermal neutrons amounted to a total of about 1%. The average neutron energy was about 1 MeV, while that of the gamma rays was 1.6 - 1.8 MeV.

The stands with the animals fixed in place, prepared for irradiation, were placed in an area located at the level of the central beam of the gamma-neutron flux. In this position, the dose field had sufficiently good uniformity. Measurements made /16
with the aid of an all-wave counter, the RUP-1 apparatus, and photodosimeters showed that the uniformity of the dose field was maintained with an accuracy of ± 10% for the neutrons and ± 5% for the gamma radiation.

The principal experiments in the study of the influence of shielding on the course and outcome of radiation damage were performed on animals which were subjected to gamma-neutron irradiation with a neutron component of approximately 90% in two dose ranges: 425 - 500 and 350 - 380 rads (Table 1). As we know, dosimetry of fission neutrons with a continuous energy spectrum poses considerable difficulties. The methods used at the present time give a rather significant error which reaches ± 20% or more. This is why, in compiling the tables, we had every reason to summarize the results of experiments using calculated doses which differ from one another by 50 rads or more, if the biological effect on the basis of the principal indices (survival rate on the 30th day and the AST of the rats which died) turned out to be the same.

TABLE 1. EFFECTIVENESS OF SHIELDING THE HEAD AND MID-SECTION OF THE STOMACH OF RATS WITH GAMMA-NEUTRON IRRADIATION AT A NEUTRON CONTRIBUTION TO THE ABSORBED DOSE OF ABOUT 90%

Radiation dose, rads	Location of shielding	Number of Rats		Survival, % ± m	AST, days M ± m
		Total	Survived		
425-500	Without shielding	70	0	0	4.9±0.2
	Shielding of head	39	1	2.6±2.5	4.5±0.2
	Shielding of stomach (2 cm)	66	4	6.1±2.9	10.6±0.5
	Shielding of stomach (4 cm)	60	20	33.3±6.1	14.8±1.3
350-380	Without shielding	30	7	23.3±7.7	8.4±0.7
	Shielding of head	30	9	30.0±8.4	9.8±0.9
	Shielding of stomach (2 cm)	28	15	53.6±9.4*	12.5±2.0
	Shielding of stomach (4 cm)	20	18	90.0±6.7*	16.0
	(Radiation control)				

*$p < 0.02$.

At radiation doses of 425 - 500 rads, all of the control animals died in an average of 4 - 5 days following the exposure, which indicates a very serious degree of radiation damage with death of the rats primarily due to the development of an acute intestinal syndrome. Under these experimental conditions, shielding of the head was practically ineffective, while slight

protection of the stomach produced an insignificant effect. It produced an increase ($p < 0.001$) (which was reliable in terms of the irradiation control) in the AST of the animals which died with the same survival rate of the protected animals. It was only with large protection of the stomach that a definite protective effect was achieved: survival of about 1/3 of the irradiated animals and an increase of the AST of the rats which died by a factor of 2.

At lower radiation doses (350 - 380 rads) shielding the /1
stomach turned out to be more effective. It produced a reliable increase relative to the controls and the survival rate of the animals: by a factor of 2 with small protection and more than 2 with large protection of the stomach. At the same time, however, shielding of the head essentially had no significant protective effect.

The rapid death of the overwhelming majority of the rats at higher radiation doses did not enable us to follow systematically the changes in the clinical picture of radiation sickness, the body weight, and indices of the peripheral blood with different localization of the protective shields. The most complete information on the changes in these indices listed above in all groups of animals was obtained in one of the experiments with gamma-neutron irradiation of rats at a dose of 380 rads.

In this experiment, in the control rats and the animals with small protection of the stomach following irradiation, there was a steady decrease in the weight for 25 days (Figure 1). During this period of time, the body weight dropped respectively by 18 and 14%. On the 30th day, the control rats showed a body weight that remained at the same level, while in the rats with small protection of the stomach there was a tendency toward recovery.

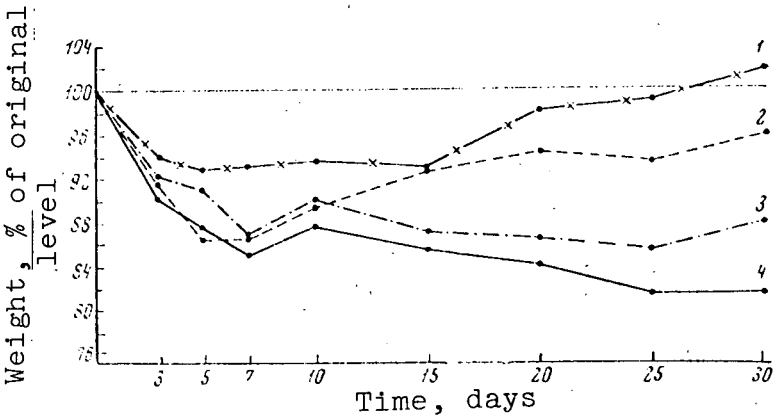

Figure 1. Body weight of rats at various times following total gamma-neutron irradiation at a dose of 380 rads (contribution of neutrons to the absorbed dose approximately 90%):

1 — "large" protection of the stomach; 2 — shielding of the head; 3 — "small" protection of the stomach; 4 — radiation control

The nature of the weight loss curve during the early periods following irradiation with protection of the head is almost the same as for the control rats: on the 5th through 7th days, the weight dropped in both groups by 13 - 15%. However, in contrast to the control rats, the animals with shielding of the head on the 30th day following irradiation showed almost complete recovery of weight to the original level.

Finally, with considerable shielding of the stomach, the maximum weight loss was recorded on the 5th day and did not exceed 7% of the original value. On the 20th day following irradiation, there was practically complete recovery of weight in the animals of this group. /167

The nature of the curve representing the weight changes at various periods of time following irradiation agrees very well with the frequency of appearance and the degree of severity of one of the principal symptoms of the acute intestinal syndrome of diarrhea. The maximum weight loss during the early periods was observed in the control rats and in the animals with shielding of the head. In these same groups, on the third day following exposure, in 90% of the cases (18 rats out of 20) there was a clearly pronounced diarrhea. At the same time, the animals with shielding of the stomach developed diarrhea in only 5% of the cases, and this to a much less severe degree. It must be emphasized that in the protected animals the diarrhea rapidly ceased, while in the control rats it was observed for a much longer time and frequently lasted until they died.

The study of the changes in the total number of leucocytes (Figure 2) showed that in animals of all groups, even as early as

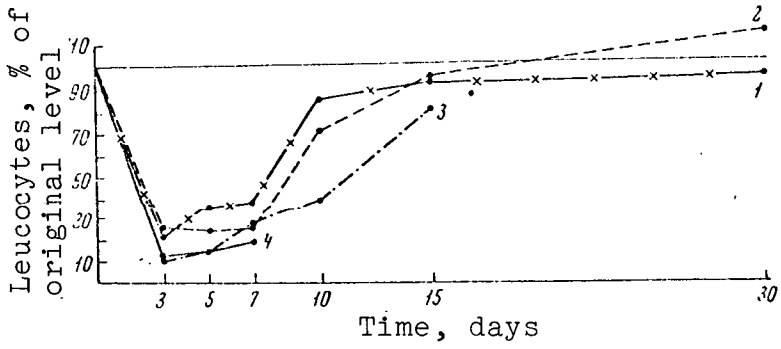

Figure 2. Number of leucocytes in the peripheral blood of rats at various periods following total gamma-neutron irradiation at a dose of 380 rads (neutron contribution to the absorbed dose approximately 90%);

Symbols the same as in Figure 1

the third day following irradiation, pronounced leucopenia had developed, but the decrease in the number of leucocytes in the control rats in the animals with slight protection of the stomach was considerably greater than in the groups with shielding of the head and considerable protection of the stomach.

In the control rats, the low level of the number of leucocytes was maintained up until the time of death in the majority of the animals (4 - 5 days). In the group with protection of the head, leucopenia remained at the same level up to 7 days, after which there was a rapid recovery of the number of leucocytes. In the rats with a small protection of the stomach, slow recovery of the number of leucocytes began on the 5th - 7th days, while in the group with considerable protection of the stomach, on the 5th day one could already see pronounced signs of recovery. On the 10th day, in this same group, the number of leucocytes /168
had already reached 85.5%, while in the group with shielding of the head it was 72.2%. The rats with slight protection of the stomach showed only approximately 41% of the original level. After 15 days, those groups with considerable protection of the stomach and protection of the head showed complete recovery of the total number of leucocytes. It also essentially returned to normal in those rats with slight protection of the stomach (83% of the original value).

Consequently, the earliest and fastest recovery of the total number of leucocytes took place in those animals which were irradiated with considerable protection of the stomach. Recovery of this index started somewhat later with shielding of the head. With slight protection of the stomach, recovery of the total number of leucocytes was delayed by approximately a week.

An analysis of the morphological state of the white blood gave the following results. The curve representing the changes in the number of neutrophils during the early stages of observation (3rd through 7th days following exposure) completely reflects the dynamics of the changes in the total number of leucocytes. In all groups, by the third day, there was already a sharp decrease in the number of leucocytes, up to 3 - 9% of the original level. Pronounced lymphopenia was retained up to the 7th day.

The recovery of the number of neutrophils and lymphocytes took place nonuniformly: the rate of recovery of neutrophils led the rate of recovery of lymphocytes. In those animals in which the head was shielded and there was considerable protection of the stomach, the recovery of the number of neutrophils took place by the 10th day, while the number of lymphocytes did not return to normal even by the end of the observation period. In the group with small protection of the stomach, the recovery of these indices of the white blood proceeded more slowly. The small number of animals which survived by the end of the observation period in groups with shielding of the head and small protection of the stomach makes a detailed analysis of the recovery of the white blood more difficult.

The reaction of the red blood at the early stages of observation was nearly the same in all groups of animals. It was characterized by a gradual decrease in the number of erythrocytes and hemoglobin.

In groups with shielding of the head and considerable protection of the stomach, on the 7th day the number of erythrocytes had dropped to 65 - 70% of the original value and remained at this level until the end of the observation period. In those

rats with small protection of the stomach, the decrease in the number of erythrocytes lasted up to 15 days and reached 48 - 49% of the original value. By the end of the observation period, this group showed an increase in the number of erythrocytes, but inasmuch as these data were obtained from only 2 rats, it would be very difficult to draw any conclusions regarding the nature of the recovery of the red blood during later stages following irradiation. Changes in the quantity of hemoglobin in all groups essentially reflected the dynamics of the changes in the number of erythrocytes. /169

The number of reticulocytes in all of the irradiated rats decreased sharply on the third day and reached a total of only 1 - 4% of the original level. However, in the protected animals, by the fifth day there was already a rapid increase in the number of reticulocytes in the peripheral blood, which was most intensive with considerable protection of the stomach and least intensive with slight protection. By the 10^{th} to 15^{th} days, the number of reticulocytes was usually 2 - 3 times greater than the original level. In the control rats on the 5^{th} day, the reticulocytes were not found in blood smears. However, beginning on the 7^{th} day in this same group, the surviving rats showed beginning regeneration of the red blood, but it proceeded much less intensively than in those animals which had the head or stomach shielded.

The experiments which were performed showed that protection of individual parts of the body of the rat, especially the area of the stomach, during exposure to gamma-neutron irradiation with a neutron component of approximately 90% leads to an increase in the radioresistance of the animals, which is most clearly evidenced in the increased survival rate and increase in the AST of the rats which died in comparison with the control animals.

However, an elementary comparison of the results obtained with the data from our previous studies that were carried out with gamma irradiation of animals [2, 3] showed that shielding the stomach during irradiation with neutrons is less effective than shielding with gamma irradiation. This is why the assumption arose that — when the percentile contribution of neutrons to the total dose of gamma-neutron irradiation of animals was decreased — the effectiveness of shielding would increase. To test this assumption, we performed a comparative experiment to evaluate the effectiveness of shielding with two modes of gamma-neutron irradiation: the first was with a neutron contribution to the dose of about 90%, and the second was with a neutron contribution of about 35% to the dose.

Inasmuch as in the second mode of irradiation there was a sharp decrease in the contribution to the total dose of the most biologically effective component of radiation from the reactor — namely, the neutrons — we felt it was necessary to increase the total radiation dose in the second version of the experiment in comparison with the dose in the first version. However, it was very difficult to predict the level of this dose. This is why we used three doses for irradiation — 500, 580,and 690 rads, increasing the dose of radiation to the animals in the first mode (350 rads) by approximately 1.4, 1.7 and 2 times.

The results of the experiment are shown in Table 2.

First of all, it is necessary to point out that when rats are irradiated with a dose of 350 rads (neutron contribution approximately 90%), the biological effect was greater than we expected. In earlier experiments, performed in 1966 - 1967, the same effect was observed when rats were irradiated with

TABLE 2. COMPARATIVE EFFECTIVENESS OF SHIELDING CERTAIN AREAS OF THE BODY OF THE RAT WITH GAMMA-NEUTRON IRRADIATION AT TWO VALUES OF THE NEUTRON CONTRIBUTION TO THE ABSORBED DOSE**

Neutron contribution to dose %	Dose, rads	Location of shielding	Number of rats		Survival % ± m	AST of rats which died, days, M ± m
			Total	Survived		
90	350	Without shielding	30	0	0	4,5±0,2
		Shielding of the head	10	0	0	4,9±0,5
		Shielding of the stomach (2 cm)	29	1	3,5±3,4	9,5±0,9
		Shielding of the stomach (4 cm)	10	2	20,0±12,6	14,3±0,9
35	500	Without shielding	10	0	0	7,0±0,6
		Shielding of the head	10	6	60,0±15,5*	9,5±2,2
		Shielding of the stomach (2 cm)	10	2	20,0±12,6*	14,0±1,9
		Shielding of the stomach (4 cm)	10	9	90,0±9,5	30,0
	590	Without shielding	20	0	0	5,1±0,6
		Shielding of the stomach (2 cm)	19	2	10,5±7,1	15,4±1,6
	680	Without shielding	10	0	0	4,1±0,1
		Shielding of the head	10	0	0	5,7±0,8
		Shielding of the stomach (2 cm)	9	0	0	8,6±1,0
		Shielding of the stomach (4 cm)	10	1	10,0±9,5	13,3±3,0

*The difference is not reliable; in the other groups it is reliable.

**Commas represent decimal points.

higher doses. It is not surprising that with such severe damage the effectiveness of even the large protection of the stomach turned out to be slight[1].

Nevertheless, the principal goal of the experiment was achieved. An analysis of the results which were obtained under the influence of gamma-neutron flux with a contribution of the neutrons to the absorbed dose of approximately 35% showed that — regardless of the increase in the total radiation dose by approximately a factor of 1.5 (up to 500 rads) — the biological effect was considerably less than with irradiation of the animals at a dose of 350 rads, with a contribution of neutrons to the absorbed dose of approximately 90%.

When the absorbed dose was increased by a factor of 1.7 (up to 590 rads), the biological effect was still less pronounced than in the first version of irradiation at a dose of 350 rads, but when the dose was increased to 680 rads the damage was slightly more serious. This was related primarily to shortening to 4.1 days the AST of the rats which died.

The results we obtained allow us to conclude that with gamma-neutron irradiation of animals the decrease in the contribution of neutrons to the total dose from 90 to 35% (with a corresponding increase in the contribution of gamma-rays from 10 to 65%) enables us to obtain a considerably higher protective

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[1]The absolute values of the survival indices and the AST of the animals which died in certain groups within a given experiment must be treated with care, since they were obtained for a comparatively small number of animals.

effect for shielding not only with uniform absorbed doses, but also with an increase in the dose when the contribution of neutrons decreases by at least a factor of 1.5. It is only when the dose is increased by a factor of 2 that the biological effect is slightly less pronounced than with irradiation at a dose of 350 rads and a higher contribution of neutrons, and the differences in the protective effect of shielding level off.

The result obtained in this experiment may be explained only by the higher effectiveness of shielding for gamma irradiation in comparison with that for neutron irradiation. This conclusion is still more convincing when we compare the effect of shielding of the stomach in rats which are subjected to radiation by neutrons and gamma rays in biologically equivalent doses.

According to the data from our previous studies, for a neutron flux dose (more exactly, gamma-neutron irradiation with a neutron component of about 90%) equal to 425 - 500 rads, the biologically equivalent dose of gamma radiation exceeds 1,000 r, inasmuch as at this dose the AST of the rats which died amounts to about 8 days. As a matter of fact, such a dose is close to 1200 r, at which all irradiated rats die in an average of 4.8 $\pm$ 0.4 days. At gamma radiation doses of 1000 - 1200 r, greater protection of the stomach insured survival of almost all irradiated animals (96.7 - 100%), while when they were irradiated with neutrons at doses of 425 - 500 rads the survival rate for the protected rats was 3 times less (33.3 $\pm$ 6.1%). Consequently, greater protection of the stomach in irradiation of /172
rats by a flux of neutrons in lethal doses is considerably less effective than with gamma irradiation in biologically equivalent doses.

A still greater difference is found when comparing the influence of slight stomach protection on the increase in survival of animals with gamma irradiation in doses of 1000 - 1200 r and the influence of neutrons at doses of 425 - 500 rads. The survival of rats in the first case reaches 85 - 90%, and in the second (as we can see from Table 1) a total of only 6.1 ± 2.9%. Consequently, at absolutely lethal doses of gamma-neutron radiation with a predominant contribution of neutrons to the dose, slight protection of the stomach was insufficiently effective, while in the case of gamma irradiation in a biologically equivalent dose it is a highly effective method of increasing radioresistance of animals.

At lower doses of gamma-neutron radiation with the predominant contribution of neutrons to the absorbed dose, the effectiveness of slight stomach protection increases noticeably, although it does lag behind (apparently) the effect of shielding with gamma irradiation.

It seems interesting to us, in concluding this report, to compare the effectiveness of shielding various masses of tissue of the head and stomach under the influence of various forms of ionizing radiation, using for this purpose some data that are presented in the present paper and also published in other works [2, 3].

It is true that we can make only an approximate comparison, inasmuch as we do not have at our disposal completely equivalent data obtained for various forms of irradiation. This applies most of all to the nonuniform values of the irradiation dose which was used in the various experiments. A more precise representation of the comparative effectiveness of shielding for various forms of irradiation may be obtained only by comparing

results which were obtained under the influence of these irradiations in biologically equal doses.

Table 3 gives some data on the effectiveness of shielding various masses of tissue of the head and middle section of the stomach under the influence of gamma rays, protons with energies of 120 MeV, and fission neutrons with average energies of 1 MeV (more accurately, gamma-neutron irradiation with a neutron component of approximately 90%).

It is obvious from the table that the results of the experiments in which the rats were subjected to the action of neutrons in doses of 350 - 380 rads and protons in doses of 800 - 1000 rads were essentially the same, regardless of the considerable difference in the radiation attenuation by the shielding used in these experiments. This result was not unexpected, inasmuch as we had determined previously [3] that — when animals are irradiated in a range of lethal doses which even slightly exceeds the minimum absolute lethal dose — highly effective shielding of the stomach is observed in those cases when even a four-fold /173
attenuation of the radiation is achieved by the protective shielding. This view is definitely supported by that part of Table 3 in which the effectiveness of shielding the stomach for gamma radiation at a dose of 1000 r and various radiation attenuation coefficients by a shielding are shown. At all the values used for this coefficient, the survival of the animals was essentially uniformly high (85 - 90%).

In experiments involving the action of neutrons and protons in the control groups, the survival rate amounted to about 25% ($LD_{75/30}$), while the AST of the rats which died was about 8.5 /174
days. Shielding the head during general irradiation with protons and neutrons in the dose ranges used was found to be ineffective.

TABLE 3. EFFECTIVENESS OF SHIELDING EQUAL MASSES OF TISSUE OF THE HEAD AND MID-SECTION OF THE STOMACH UNDER THE INFLUENCE OF DIFFERENT TYPES OF IONIZING RADIATION

Type of radiation and dose	Degree of radiation attenuation by shielding	Shielding location	No. of rats		Survival, % ± m	AST, rats which died, days, M ± m
			Total	Survived		
Gamma-neutron flux, neutron contribution to dose approx. 90%, E_n=~1 MeV, 350-380 rads	Neutron flux by 4.6; gamma rays by 2.3 times	Without shielding	30	7	23.3±7.7	8.4±0.7
		Shielding of head	30	9	30.0±8.4	9.8±0.9
		Shielding of stomach (2 cm)	28	15	53.6±9.4	12.5±2.0
Protons, E=120 MeV, 800-1000 rad	By 10 - 12 times	Without shielding	24	6	25.0±8.8	8.5±0.7
		Shielding of head	24	4	16.6±7.6	7.4±0.7
		Shielding of stomach (2 cm)	24	12	50.0±10.2	11.4±1.8
Gamma rays, 1000 r	By 25 - 30 times	Without shielding	221	4	1.8±0.9	7.9±0.2
		Shielding of head	30	9	30.0±8.4	8.2±1.2
		Shielding of stomach (2 cm)	48	41	85.4±5.1	16.3±3.2
	By 12 times	The same	48	43	89.6±4.4	19.2±2.4
	By 4 times	The same	47	40	85.1±5.2	14.1±1.8

At the same time, however, shielding the same mass of tissue in the middle portion of the stomach increased the survival rate of the rats in comparison to the controls by a factor of 2.

Unfortunately, we do not have any data on the effectiveness of shielding with gamma irradiation at a dose equivalent in its biological effect to the use of doses of neutrons and protons. The table contains data obtained for a dose which causes death in 98% of the irradiated control rats ($LD_{98/30}$). Nevertheless, even at this dose of gamma rays, whose biological effect is considerably higher than with the doses of neutrons and protons which were employed, shielding the head led to a considerable increase in the survival rate of the animals, which we did not observe under the influence of neutrons and protons, while shielding the same mass of stomach tissue produced a higher survival rate of rats than in the studies mentioned above: 85 - 90% instead of 50 - 55%.

Hence, in our experiments involving irradiation of rats with protons with energies of 120 MeV and fission neutrons with an average energy of approximately 1 MeV, shielding of the head did not have a significant influence on the outcome of radiation damage: the survival rates of the protected and control animals were essentially the same. However, on the basis of certain indices of radiation damage — for example, the change in body weight and the number of leucocytes — we can detect a positive influence of shielding the head during total irradiation with neutrons and protons. Thus, in those rats which had the head protected, in contrast to the control animals, the body weight was almost normal by the 30^{th} day after exposure to neutrons. In this same form of irradiation, shielding of the head, although it did not prevent an initial decrease in the leucocyte count, markedly accelerated its recovery.

Under exposure to the action of biologically equivalent doses of neutrons and protons, the effectiveness of a slight protection of the stomach was the same — the survival rate of the animals increased by a factor of 2 in comparison to the irradiated controls.

Hence, shielding portions of the midsection of the stomach under the influence of protons and neutrons leads to a considerable increase in the radioresistance of the animals (rats), and shielding the same mass of tissue in the head has only a slightly positive effect. With gamma-irradiation, shielding the head produces a considerable increase in the radioresistance of the animal; it not only accelerates the recovery of body weight and the principal blood indices, but also markedly increases the survival rate of the animals. Slight protection of the stomach in gamma irradiation of rats leads to a sharply pronounced increase in their radioresistance and insures a high degree of survival of the animals at lethal doses of radiation.

Our studies showed that shielding of certain parts of the /17
body, especially portions of the midsection of the stomach — under the influence of fission neutrons with an average energy of about 1 MeV and protons with an energy of 120 MeV — increases the radioresistance of the irradiated animals. However, shielding when these forms of radiation are applied is less effective than shielding for gamma irradiation.

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Change in Reactivity of Animals to Certain Pharmacological Preparations With Parts of the Body Shielded During Total Irradiation

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ABSTRACT. In experiments on rats, the authors studied cystamine, AET and 5-methoxytryptamine with shielding of the stomach and head under conditions of general irradiation with protons with energies of 120 MeV and gamma rays from Co^{60}. The effectiveness of these preparations was considerably increased, especially when the substances were used in doses that were reduced relative to the optimum together with "slightly effective" shieldings, with absolutely lethal doses of radiation on the animals. While in the case of cystamine and AET an increase in the specific effectiveness was the same, both for shielding the head and for the tissue of the stomach, in the case of 5-methoxytryptamine with shielding of the stomach the increase in its effectiveness was much more pronounced than with shielding of the head. Such differences in the reaction of the animals are obviously explained by certain differences in their action.

During the last 20 years, since the publication of the /175
first papers by Patt [9, 10], Bacq [4, 5] and Jacobson [6, 7], a very great many studies have been devoted to investigating the possibility of increasing the radioresistance of animals by means of chemical substances administered to the organism prior to exposure or by shielding individual, highly radiosensitive organs and tissues during irradiation.

Shortly thereafter, Jacobson et al. [8] showed that the radioresistance of mice that were irradiated with shielding of the spleen increases to a greater degree if they receive

estrogen or cysteamine prior to exposure. A similar result was obtained by Mezen et al. [1] in experiments on rats, which had received cystamine and were shielded during irradiation with x-rays in the region of the liver or certain parts of the stomach.

The present paper describes results of experiments on the influence of shielding the stomach and head on the change in reactivity of the animals (rats) with respect to certain pharmacochemical substances with total irradiation by protons and gamma rays from Co^{60}. The experiments were performed on white, random-bred, sexually mature rats of both sexes. The observation period of the animals following irradiation was 30 days. The observed effect was evaluated based on the indices of survival for 30 days following irradiation and the average survival time (AST) of the animals which died.

The animals were irradiated in a fixed position in special holders. For irradiating the animals with a flux of protons, we used the synchrocyclotron of the Joint Institute of Nuclear Research at Dubna, which emits a narrow beam of protons with an energy of 657 ± 5 MeV in the form of pulses lasting 100 - 400 microseconds at the rate of 100 impulses per second. By means of polyethylene blocks and lead sheets, we succeeded in reducing the energy of the protons to 120 MeV and in expanding to a certain degree the effectively usable cross section of the beam, which made it possible to increase the number of animals that could be irradiated simultaneously and also made it possible to decrease considerably the differences in the doses in various parts of the irradiation field. However, the pulsed nature of the proton flux and the oscillations in its intensity as well as certain errors in dosimetry of the protons led to a significant scatter of the readings for the dose levels of radiation which

were received by the animals in various parts of the field and in different batches. Therefore, we had to combine into single experimental groups the animals which had received (according to dosimetric data) slightly different doses of radiation but which showed practically the same picture of radiation damage in the control series. The intensity of the dose of protons with E = 120 MeV was 60±10 rads per minute.

Gamma irradiation of the animals was performed with an experimental irradiating device with a Co^{60} charge at two dose level ranges: 10.7 - 13.5 and 33.7 - 38.8 r/min. The use of the gamma irradiator made it possible to reproduce accurately all irradiation conditions and to measure the dose of gamma rays not only in each batch, but also in each successive experiment in the same series. The method of performing the experiment and irradiating the animals is described in greater detail in a paper published earlier by B. L. Razgovorov et al. [2].

When irradiating the rats with protons, we used blocks for shielding the region of the stomach which were made from plastic (polymethylmethacrylate), 6, 4, and 2 cm wide and 15 cm thick. When the head was shielded, the width of the blocks was increased to 8 - 10 cm. Plastic blocks 15 cm thick reduced the dose of radiation by 10 - 12 times, so that the residual dose behind the shielding did not exceed 8 - 10% of the exposure dose.

With gamma irradiation, the shielding of parts of the body was accomplished with the aid of steel sheets 3 cm thick. When shielding portions of the stomach area, the width of the block was 2 cm, while when the head was shielded it was 8 - 10 cm. Steel shields 3 cm thick reduced the dose of gamma rays by 2.5 times, so that the residual dose behind the shielding was 40% of the exposure dose. /177

When shielding the head, it was found that the skull and its contents were protected, as well as the three anterior cervical vertebrae and the distal portions of the anterior extremities (forelegs and paws). The mass of all the protected tissues amounted to about 13.5% of the weight of the rat.

In rats weighing about 200 g, fixed in a stand for irradiation on their backs, the distance from the xiphoid process on the chest to the pubic joint was usually about 8 cm. For a more precise designation of the location of the protective blocks during irradiation, we shall conditionally divide the area of the stomach into four transverse segments, each 2 cm wide, beginning with the level of the xiphoid process, and call them the first, second, third and fourth segments.

When protecting the area of the stomach with a block 6 cm wide, the latter shields the first three segments, whose mass amounts to about 35% of the weight of the rat. In this case, the liver, spleen, both kidneys with adrenals, stomach with duodenum, the principal mass of loops of intestine, the two posterior ribs (completely) and the two following ribs (partly), and about 10 vertebrae (4 posterior spinal and all 6 lumbar) are protected.

When shielding the area of the stomach with a block 4 cm wide, the organs and tissues of the second and third segments were protected: more than half (about two-thirds) of the spleen, and a considerable portion of the stomach, the principal mass of the loops of intestine, the entire left kidney with adrenal and the lower half of the right kidney, the 6 lumbar vertebrae. The mass of the protected tissue was approximately 24%. Finally, when a block 2 cm wide was used, the latter was usually placed in the vicinity of the second and third segments, shielding the

central portion of the stomach. The block then protected approximately half the spleen, a portion of the loop of the intestine, both kidneys partially, and 3 lumbar vertebrae. The total mass of protected tissue was above 11.5 - 12%.

In the experiments, we used three preparations: cystamine dichlorhydrate, S-,β-aminoethyisothiuronium bromide hydrobromide (AET) and 5-methoxytryptamine (5-MOT). AET was used in combination with cystamine in different amounts. The doses were calculated on the basis of the compounds used. The substances were dissolved in distilled water immediately prior to the experiment and administered intraperitoneally at the rate of 2 ml of solution per rat weighing 200 grams, 15 - 20 minutes prior to exposure. This interval was governed by the time required for fixation and movement of the rats in the stands for irradiation.

During the first experiments which were performed in 1962 - 1964, the animals were subjected to the action of protons with energies of 120 MeV in doses of 750 - 1100 and 1100 - 1300 rads. Prior to irradiation, the rats received intraperitoneal injections of cystamine and AET at the rate of 50 mg/kg of each substance[1], while during the irradiation process of the area of the stomach was protected: in the majority of experiments, the blocks used were 6 cm wide and it was only in the last two experiments that they were 4 and 2 cm wide. The total results of this series are shown in Table 1. /178

(1) The joint application of preparations at these doses led in some experiments to individual cases of toxic death of the animals.

TABLE 1

SURVIVAL AND AVERAGE LIFETIME OF EXPERIMENTAL AND CONTROL RATS WITH IRRADIATION BY PROTONS AT AN ENERGY OF 120 MeV

Shielding	Dose, rads	No. of Rats	Survival		AST of rats which died (M±m), days
			Absolute	%±m	
Without shielding	750 — 1100	114	18	15.8±3.4	8.0+0.4
	1100 — 1300	66	5	7.6±3.3	7.4±0.6
Cystamine + AET, 50 mg/kg of each	750 — 1100	110	57	51.8±4.8*	13.1±0.8
	1100 — 1300	60	22	36.6±6.2*	10.7±0.8
Shielding of the stomach (6 cm)	750 — 1100	96	81	84.4±3.7**	13.5±1.7
	1100 — 1300	75	49	65.3±5.5**	12.7±0.9
Cystamine + AET + shielding of the stomach (6 cm)	750 — 1100	97	85	87.6±3.4**	13.7±2.1
	1100 — 1300	63	56	88.9±4.0***	10.0±1.6
Shielding of the stomach (4 cm)	800 — 1050	36	28	77.8±6.9***	8.8±2.3
Cystamine + AET + shielding of the stomach (4 cm)	800 — 1050	36	35	97.2±2.8***	12.0
Shielding of the stomach (2 cm)	800 — 1000	24	12	50.0±10.2*	11.4±1.8
Cystamine + AET + shielding of the stomach (2 cm)	800 — 1000	24	17	70.8±9.3*	14.8±3.3

(Footnotes appear on following page).

TABLE 1 (Footnotes)

Note: The differences are statistically reliable:

* With respect to irradiation control

** With respect to irradiation control and mixture of preparations.

*** With respect to irradiation control, mixture of preparations and shielding.

As we can see from the table, at a radiation dose which causes death in 85% of the irradiated animals in the control group ($LD_{85/30}$), the mixture of cystamine and AET increased the survival rate of the rats in comparison to the control group by 35% on the average, while shielding the portion of the stomach by a block 6 cm wide increased it by almost 70%. In both cases, the increase in the survival rate was statistically reliable ($p < 0.001$). Joint application of these preparations and protection (at this dose of radiation) gave essentially the same effect (based on survival of rats) as a single shielding of the area of the stomach.

At a higher radiation dose ($LD_{92-93/30}$) the effectiveness of both the mixture and shielding of the stomach turned out to be somewhat less than in the preceding version of the experiment, although in this case the increase in survival rate in comparison to the control group of animals was statistically reliable ($p < 0.001$). At the same time, however, with the joint application of a mixture of preparations and shielding of the stomach with a block 6 cm wide, the survival rate of the animals at a radiation dose of 1100 - 1300 rads was equally as high as with a smaller dose of irradiation. It is important to note in this regard that a statistically reliable increase in the index of survival ($p < 0.001$) is observed not only in comparison with the control group, but also in comparison with the groups

of animals which received either preparations or shielding of the stomach area separately.

At a radiation dose of 750 - 1100 rads, the decrease in the width of the blocks leads to a drop in the shielding effect, and is especially noticeable when the block width is 2 cm. At the same time, however, the joint application of cystamine and AET with shielding of the stomach by a block 4 cm wide is no less effective than when using the same preparations together with a shielding 6 cm wide. It is only when the width of the shield is decreased to 2 cm that the joint application of chemical substances and shielding is less effective, although in this case it still remains rather high: the survival rate of the protected rats amounts to about 75% and exceeds by approximately 20% the same index for separate use of a mixture of cystamine and AET and shielding of the stomach.

The experiments performed indicate that shielding of the stomach area in rats during total irradiation with protons changes the reactivity of the animals with respect to cystamine and AET administered prior to irradiation: the effectiveness of their action, evaluated on the basis of the survival of the animals with shielding of the stomach, is higher than without /180
shielding at the same doses of administered substances. The observed effect is more clearly evident both with an increase in the radiation dose and with a decrease in the shielding width (naturally, to a certain degree). However, the method of shielding which we have used in these experiments, which itself ensures a sufficiently high survival rate of animals at the selected doses of radiation, essentially "complicated" the development of the given effect. Therefore, the last experiments were performed on animals subjected to gamma radiation using shieldings of slightly different shape.

TABLE 2

SURVIVAL AND AVERAGE LIFETIME OF EXPERIMENTAL AND CONTROL RATS WITH GAMMA IRRADIATION AT A DOSE OF 900 r

Protection	Number of Animals	Survival		AST of rats which died (M±m), days
		Absolute	%±m	
Without shielding	20	0	0	9.4±0.9
Shielding of the stomach	18	10	55.6±11.7	14.8±1.7
Cystamine, 75 mg/kg	20	12	60.0±11.0	15.9±2.0
Cystamine, 25 mg/kg	20	3	15.0±8.0	12.0±1.5
Cystamine, 25 mg/kg + shielding of the stomach	20	19	95.0±4.9*	16.0
5-MOT, 5 mg/kg	20	1	5.0±4.9	10.9±1.0
5-MOT, 5 mg/kg + shielding of the stomach	18	17	94.4±5.4*	17.0

* $P < 0.05$ for the corresponding preparation and shielding of the stomach

In the experiments [3] we established the dependence of the shielding on the mass of protected tissue and the attenuation of the dose by the shielding. These studies made it possible to select shieldings with dimensions such that their use during irradiation of the animals with lethal doses of gamma radiation would give a relatively slight effect (judged by survival by the 30^{th} day). In particular, for protecting the stomach, we used steel blocks 2 cm wide and 3 cm thick, whose residual dose was large, 40% of the exposure dose.

In the first series of experiments, the animals were subjected to total gamma irradiation at a dose of 10.7 - 13.5 r/min. It was only in the first experiment of this series that the rats received a radiation dose of 900 r, which under these conditions was the absolutely minimum lethal dose, while in the other experiments the radiation dose was increased to 1000 r.

In the first experiment, we used two preparations - cystamine and 5-methoxytryptamine, while in the other experiments we used cystamine as well as cystamine combined with AET in doses of 50 and 25 mg/kg of each substance. The results of the experiments are shown in Tables 2 and 3.

The data in Table 2 show that intraperitoneal administration /181 of cystamine to rats at a dose of 75 mg/kg ensured survival of 60% of the irradiated animals. When the dose of the preparation was reduced by a factor of 3 (to 25 mg/kg), only individual animals survived. The same effect was observed when rats received 5-MOT at a dose of 5 mg/kg. With joint use of shielding of the stomach and cystamine at a dose of 25 mg/kg or 5-MOT in a dose of 5 mg/kg, we observed a sharp increase in the effectiveness of both preparations - up to 95% of the irradiated animals

TABLE 3

SURVIVAL AND AVERAGE LIFETIME OF EXPERIMENTAL AND CONTROL RATS WITH GAMMA IRRADIATION AT A DOSE OF 1000 r

Protection	Number of Animals	Survival		AST of rats which died (M±m), days
		Absolute	%±m	
Without shielding	138	1	0.7±0.7	8.1±0.2
Shielding of the stomach	137	37	27.0±3.8	12.9±0.5
Cystamine + AET (50 mg/kg)	17	12	70.6±11.1	21.2±3.3
Cystamine + AET (50 mg/kg) + shielding of the stomach	21	20	95.2±4.7*	12.0
Cystamine + AET (25 mg/kg)	48	3	6.3±3.5	10.2±0.6
Cystamine + AET (25 mg/kg) + shielding of the stomach	48	29	60.4±7.1**	18.6±1.4
Cystamine (50 mg/kg)	60	2	3.3±2.3	10.3±0.5
Cystamine (50 mg/kg) + shielding of the stomach	60	38	63.3±6.2**	15.7±1.5
Cystamine (25 mg/kg)	113	3	2.7±1.5	9.5±0.3
Cystamine (25 mg/kg) + shielding of the stomach	120	59	49.1±4.6**	12.9±0.6

* The difference is statistically reliable only for shielding of the stomach.

** The difference is statistically reliable for both shielding of the stomach and for preparations at this dose.

survived. It is necessary to point out that the observed effect (on the basis of the survival index) was higher than the figures obtained with combined survival indices of rats with separate application of shielding of the stomach (about 55%) and the preparations mentioned above (15 and 5%).

However, when rats were exposed to gamma radiation at a dose of 900 r, the shielding which we employed provided still too high a survival rate of the animals, so that in all subsequent experiments in this series the radiation dose, as we have already said, was increased to 1000 r. At this dose, the intraperitoneal administration of a mixture of cystamine and AET to rats (50 mg/kg of each preparation) provided a sufficiently high survival effect (about 70%). Shielding the stomach reliably ($p < 0.001$) increased the effect of using the /182
mixture, but the result obtained (on the basis of survival) was essentially equal to the sum of the survival indices for separate application of the mixture and shielding of the stomach.

The decrease in the dose to 25 mg/kg led to a sharp drop in the effectiveness of the mixture, and only individual rats survived. However, with the joint use of preparations and shielding of the stomach their effectiveness (judging by the survival rate of the rats) increased several fold and was 2 times higher than the sum of the survival indices with separate administration of the shielding and preparations at the same dose. The same result was obtained in an experiment in which — instead of a mixture of cystamine and AET with 25 mg/kg of each preparation —— cystamine alone was used at a dose of 50 mg/kg.

The administration of cystamine to the rats at a dose of 25 mg/kg had practically no influence on the survival rate of

the animals: various survival indices of the experimental and control animals were unreliable. At the same time, however, the administration of the preparation in the indicated dose prior to irradiation of the rats with shielding of portions of the stomach ensured survival of half the irradiated rats. It is necessary to note that various survival indices of the rats which were obtained with joint application of shielding of the stomach and cystamine in doses of 50 and 25 mg/kg (63.3 and 49.1%) turned out to be statistically unreliable.

Experiments on rats subjected to gamma radiation supported the data which we obtained previously when using protons, on increasing the effectiveness of cystamine and AET when used with shielding of portions of the abdominal cavity.

When irradiating animals with protons in one of the latter experiments, we attempted to compare the influence on the effectiveness of a mixture of cystamine and AET produced by shielding various masses of tissue of the head and stomach, which takes place when the entire head and one of the segments of the stomach (2 cm wide) are shielded. For this experiment, we had at our disposal reliable data on the considerably lesser effectiveness of shielding the head in rats in comparison with the effect of shielding the same mass of tissue in the stomach area with lethal doses of radiation. We therefore assumed that with joint utilization of cystamine and AET (50 mg/kg each) the survival rate of the animals with shielding of the head under conditions of total irradiation in lethal doses would be much less than with utilization of the same preparations and shielding of the stomach. However, in this experiment we obtained practically the same survival rate for the rats of the compared groups. Unfortunately, due to certain circumstances,

we could not repeat this experiment with irradiation of rats by protons, and the comparatively limited number of animals that was used in this experiment prevented us from drawing any conclusions at this time prior to checking the data obtained. /183

Later, we performed a series of experiments of a similar nature, but with certain changes in the conditions under which they were performed. First of all, the animals were subjected to the action of gamma rays at much higher doses than when they were exposed to protons. If in a similar experiment 75% of the control animals died ($LD_{75/30}$) when exposed to protons, with gamma irradiation we used absolutely lethal doses: 1000 r at a dose of 10.7 - 13.5 r/min and 925 - 945 r at a dose of 33.7 - 38.8 r/min. In the first case, the radiation dose was approximately 100 r (and in the second, approximately 125 - 150 r) greater than the minimum absolute lethal dose for rats under the given conditions of exposure. In addition, for protecting the stomach and head, we used "slightly effective" blocks, behind which the residual dose reached 40% of the exposure dose. The results of the experiments are shown in Table 4.

An analysis of the data in this table will show that for gamma irradiation, just as with protons, the effectiveness of cystamine administered to rats in doses of 50 and 25 mg/kg sharply increases and turns out to be practically the same for both shielding of the stomach and the head. Under these conditions, the indices of survival of the animals of the given experimental groups are statistically reliably higher than the survival indices of all the control groups, including the groups of rats with only shielding of the stomach.

With intraperitoneal administration of 5-methoxytryptamine to rats in doses of 5 - 6 mg/kg, we obtained slightly different

TABLE 4

SURVIVAL AND AVERAGE LIFETIME OF EXPERIMENTAL AND CONTROL RATS SUBJECTED TO GAMMA IRRADIATION AT DIFFERENT DOSE INTENSITIES

Dose of Gamma radiation and dose intensity	Protection	Number of animals	Survival		AST of rats which died (M±m), days
			Absolute	%±m	
1000 r, 10.7 - 13.5 r/min	Without shielding	72	0	0	8.2±0.3
	Shielding of the stomach	66	20	30.3±5.7	13.4±0.8
	Shielding of the head	29	3	10.3±5.6	9.0±1.1
	Cystamine (50 mg/kg)	60	2	3.3±2.3	10.3±0.5
	Cystamine (50 mg/kg) + shielding of the stomach	30	24	80.0±7.3*	14.2±2.7
	Cystamine (50 mg/kg) + shielding of the head	39	32	82.1±6.1*	15.7±3.2
925-945 r, 33.7 - 38.8 r/min	Without shielding	60	0	0	5.7±0.3
	Shielding of the stomach	48	8	16.7±5.4	14.1±1.0
	Shielding of the head	48	6	12.5±4.8	6.2±0.7
	Cystamine (25 mg/kg)	36	0	0	8.8±0.4
	Cystamine (25 mg/kg) + shielding of the stomach	36	17	47.2±8.3*	13.4±1.3
	Cystamine (25 mg/kg) + shielding of the head	36	16	44.4±8.3*	10.8±1.5
	5-MOT (5-6 mg/kg)	54	2	3.7±2.6	8.8±0.6

(Continued)

TABLE 4 (Continued)

SURVIVAL AND AVERAGE LIFETIME OF EXPERIMENTAL AND CONTROL RATS SUBJECTED TO GAMMA IRRADIATION AT DIFFERENT DOSE INTENSITIES

Dose of gamma radiation and dose intensity	Protection	Number of animals	Survival		AST of rats which died (M±m), days
			Absolute	%±m	
	5-MOT (5-6 mg/kg) + shielding of the stomach	60	46	76.7±5.5*	16.3±1.8
	5-MOT (5-6 mg/kg) + shielding of the head	52	19	36.5±6.7*	10.1±1.2

* The differences are statistically reliable for both the corresponding preparations (cystamine or 5-MOT) and for shielding of the head or stomach.

results. Judging by the survival index of animals by the 30th day, shielding of the stomach and shielding of the head were statistically reliable and in comparison with corresponding control groups, increased the effectiveness of the preparation. However, this increase in its effectiveness with shielding of the stomach was approximately 2 times higher than with shielding of the head (the differences were also statistically reliable, $p < 0.001$).

The materials presented in this work reliably show that shielding parts of the body with total irradiation considerably changes the reactivity of the animals to the effects of ionizing radiation. In all experiments without exception, we noticed a pronounced increase in the radioresistance of the irradiated rats. At the same time, the experiments showed that in those animals which were irradiated with the head or stomach shielded there was also a change in the reactivity to certain pharmacochemical preparations, especially cystamine and AET (representative of mercaptoethylamines) as well as 5-methoxytryptamine (a representative of indolylalkylamine). When the animals were irradiated with portions of the body shielded, the specific effectiveness of these preparations increased markedly. This increase in effectiveness was particularly apparent in those cases when the preparations were used in reduced doses (in comparison to those generally employed - optimum) along with "slightly effective" shieldings when the animals were exposed to absolutely lethal doses of radiation.

At the same time, the method we employed in performing the /184
experiments made it possible to reveal certain differences in the protective effect with the use of preparations from various classes of compounds as a function of the location of the shielding. While an increase in the protective effectiveness

of cystamine and AET, essentially speaking, was the same both for shielding of the head and for shielding of the same mass of stomach tissue, for 5-methoxytryptamine when the stomach was shielded the protective effect was increased to a much greater degree (by approximately a factor of 2) than when the head was shielded.

Such a difference in the reaction of animals to shielding /185
of the stomach and head with administration of preparations that belong to different classes of compounds apparently is explained by certain differences in the action of mercaptoethylamines and indolylalkylamines. It is quite likely that with absolutely lethal doses of radiation — under those conditions when the shielding does not protect the region of the stomach and thereby does not have a significant influence on the damage to the intestines, which frequently is the case, especially with shielding of the head — the effectiveness of the preparations which do not have a significant protective effect on the gastrointestinal tract will be increased to a lesser degree than with shielding of the stomach.

It is possible that the method which we used for our experiments (the administration of chemical protectors to the animals prior to exposure with various parts of the body having an equivalent mass shielded) may be used for studying certain aspects of the action of preparations belonging to different classes of compounds.

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Influence of Shielding Certain Parts of the Body on the Course of Radiation Sickness in Dogs with Total Gamma Irradiation

B. L. Razgovorov and N. I. Konnova

ABSTRACT. In an experiment on dogs which were subjected to total gamma-irradiation at lethal doses, the authors studied the influence of shielding the head and the anterior part of the stomach on the clinical course and outcome of radiation sickness. The experiment results supported the basic data obtained in experiments on small laboratory animals and showed the high effectiveness of shielding a part of the body in dogs. In protecting various masses of tissue with shieldings that provided equal attenuation of the radiation and with an equal dose of radiation, shielding of parts of the anterior portion of the stomach was much more effective than shielding the head. To have high protective shielding at lethal radiation doses, it is necessary to protect the mass of the stomach tissue (head) that is equal to 10 to 15% of the mass of the body and to reduce the radiation dose to the shielded tissues by 3 to 4 times.

In our preceding studies [2, 4], performed on random-bred white rats, we showed that shielding certain parts of the body (primarily the region of the stomach) with total gamma irradiation and exposure to the action of high-energy protons at lethal doses considerably changes the course of radiation sickness. This is /186 manifested primarily in a decrease in the severity of damage and a decrease in the number of lethal outcomes. In other words, the screening of portions of the body with total irradiation of rats increases their radioresistance, i.e., it has a pronounced protective effect.

It was also established that at lethal radiation doses a high protective effect of shielding is developed under the following

two conditions: (a) the mass of protected tissue, primarily organs of hemopoiesis and the intestine, must amount to about 10 - 15% of the body weight; (b) the radiation dose of the shielded tissues must be decreased (by means of a shielding) by approximately a factor of 4. With a decrease in the attenuation of the radiation dose by the shielding up to 2.5 fold, the protective effect drops sharply.

In this paper, we present the results of an experiment which was performed on 37 mongrel dogs weighing about 12 kilograms on the average, subjected to total gamma irradiation for the purpose of studying the influence of shielding the head or stomach on the course and outcome of radiation sickness. First of all, it was necessary to answer the question of whether or not shielding parts of the body in dogs exposed to general gamma irradiation is as effective as was the case in experiments on rats, under the above-mentioned shielding conditions. In other words, it was necessary to determine whether or not our conclusions that apply to shielding conditions producing a reliable protective effect were valid for experiments on larger animals, i.e., whether they had a general biological nature.

Following preliminary examination (check-up, weighing, study of peripheral blood), the dogs were exposed to systematic training in a special capsular stand in order to accustom them to prolonged and quiet standing in a relatively fixed position, so that the duration of exposure at a dose of 600 r reached 3 1/2 hours. The condition of the animals and their behavior during training and irradiation were checked by television cameras.

The dogs used for irradiation with shielding of the stomach were dressed in a special jacket to which a protective lead block

was attached at the desired point, mounted in a vertical position on the stand and fastened to the bottom of the capsule. The block had a curvature that corresponded to the shape of the lateral surface of the stomach. Shielding of the head was performed by means of a rectangular lead block that was fastened to the stand in front of the capsular chamber in which the head of the dog was fastened.

In special studies that were performed on frozen dog corpses, it was established that the mass of tissue of the head together with the 2 - 3 anterior cervical vertebrae amounts to 11 - 13% of the weight of the entire body. By weighing individual segments of /187 the trunk, we determined the limits of the segment of the stomach, located immediately in front of the umbilical line, whose tissue mass was approximately equal to the tissue mass of the head. On the basis of the materials thus obtained, we found that for protecting such a segment of stomach it is necessary to use a shielding approximately 8 cm thick.

When shielding the above segment of the stomach, the following principal organs were protected: half or most of the spleen, 4 - 4.5 vertebrae, portions of the two posterior ribs, the right kidney and half or forward 2/3rds of the left kidney, both adrenals, a portion of the loop of the intestine (up to 1 meter or more), a portion of the stomach and parts of certain other organs and tissues.

In the experiments, we used shieldings of various thicknesses depending on the necessary attenuation of the radiation dose by the material of the protective screening. The maximum degree of attenuation (by a factor of 4) was selected by us on the basis of results obtained in a study on rats. At the same time, when shielding the stomach of dogs, we tested lesser degrees of dose attenuation (by factors of 2 and 3).

The dogs were irradiated in the capsular stand described above, mounted at a distance of 2 meters from the source of gamma radiation — the "Khizotron" apparatus with a Co^{60} source. The intensity of the dose of gamma radiation at the center of the capsule was about 2.9 r/min, while the difference between these indices at the center and at the corners of the capsule did not exceed 10%.

All four groups of dogs with protection and one control group were subjected to general gamma irradiation at a dose of 600 r. An additional group of control dogs was added to the experiment, irradiated with a dose of 500 r, which was approximately 15% less than the dose of radiation of the principal groups of animals. This was done in order to compare the effectiveness of shielding various parts of the body of the dogs in the protected groups with control animals subjected to the action of gamma radiation, not only with equal doses but also with similar approximately absorbed doses.

Following irradiation of the dogs, they were examined regularly and subjected to checks at specified periods. The dogs which survived were kept under observation for 60 days and sometimes longer. The evaluation of the shielding effectiveness was performed on the basis of an analysis of the damage, dynamics of weight, and principal blood indices.

The distribution of the animals by groups and the principal results of the experiment are shown in Table 1.

In the dogs of the first and second control groups, there was typical acute radiation sickness of the third degree of severity (III) with death of all animals in the period from the 9^{th} to the 18^{th} days following exposure. In all dogs, as a rule,

TABLE 1. SURVIVAL OF DOGS IN TOTAL GAMMA IRRADIATION WITH SHIELDING OF VARIOUS MASSES OF TISSUE OF THE HEAD OR STOMACH.

Dose, r	Degree of radiation dose attenuation by shielding	Group and Shielding	Number of Dogs		Survival, %	AST of dogs which died, days	Times of death, days after exposure
			Total	Survived			
500	-	First Without shielding	7	0	0	15.7	13, 14, 15, 16, 16, 18, 18,
600	-	Second Without shielding	6	0	0	13.7	9, 12, 12, 15, 17, 17
	4.1	Third Shielding of the head	7	4	57.0	20.3	14,20,27
	4.1	Fourth Shielding of the stomach	7	6	86.0	-	33
	3.0	Fifth Shielding of the stomach	5	4	80.0	-	10
	2.0	Sixth Shielding of the stomach	5	4	80.0	-	18
	From 2 to 4.1	Fourth - Sixth Shielding of the stomach	17	14	82.0	20.0	10,18,33

on the first two or three days there was already a deterioration of the general condition: there was a drop in activity and feeding excitement. Later on, the condition of the animals improved, but the period of simulated well-being was usually brief. Seven to 12 days after exposure, typical symptoms of the climax of the disease developed. Thus, for example, at these times all six dogs of the second group and six out of the seven dogs in the first group showed development of fluid feces. /188

In the majority of dogs of both groups, toward the end of their lives, they developed a typical hemorrhagic syndrome: admixture of blood in the feces, hemorrhaging in the mucous and submucous linings of the oral cavity, and sometimes the eyes, hemorrhagic flows from the oral cavity and nose. In four dogs there was also edema of the muzzle, especially pronounced in the area of the lower lip and lower jaw; there was formation of extensive hematomas in the subglossal region. All animals during the last days of life showed a drop in weight but it was not so severely pronounced (approximately 10%) in conjunction with their rapid death. /189

In the third group of seven dogs, three died. In two of them we found radiation sickness of acute degree with about the same essential clinical picture as in the control animals. Beginning with the first few days, we noticed a deterioration of the general condition, a drop in activity and appetite and following a brief period of improvement from the 7^{th} through the 9^{th} days there were fluid feces, signs of hemorrhagic syndrome, etc. Toward the end of their lives (14 through 20^{th} days) the weight of the animals dropped by 25 - 26%. In the third dog, which died on the 27^{th} day from acute radiation sickness, the marked and rapidly progressive deterioration of condition began only toward the end of the second week, but there were no clear external signs of the

development of hemorrhagic syndrome in this animal even at the end of its life. At the same time, however, by the third week after exposure this dog already showed a marked drop in the indices of hemoglobin and erythrocytes — up to 20 - 25% of the original level, while the weight loss at the end of its life was 15 - 16%.

In the four dogs which survived, there was a milder course of radiation sickness, although in three of them there was a typical diarrhea in a severely pronounced form. The hemorrhagic syndrome in the dogs with shielding of the head was less clearly evident than in the control animals. In only one dog did we observe a sanious excretion from the nose, while this dog like five others showed damage to the gums and periodical development of slight hemorrhages in the mucous membranes of the oral cavity. In those dogs which survived in the third group, the body weight by the 30^{th} day had reached only 89% of the original level and 96% on the 60^{th} day.

In dogs with shielding of the stomach, in all three groups we noticed a milder radiation sickness, especially with use of protective shielding with four-fold attenuation of the radiation dose.

In the fourth group only one out of seven dogs died, on the 33^{rd} day after exposure. For more than three weeks, the state of the animal was completely satisfactory. However, toward the end of the month the activity of the dog slowed down and it began to lose weight rapidly. At the same time, with good recovery of the number of leucocytes, there was a rapid drop in the number of erythrocytes and hemoglobin with total absence of any kind of disruptive signs in the hemorrhagic diathesis. On autopsy, we found a cardiac tamponade: the cavity of the pericardium contained

approximately 250 milliliters of fluid blood with admixture of clots.

In the remaining dogs of the fourth group, we noticed a mild radiation sickness. Only three of the animals occasionally showed liquid and semi-liquid feces; there were no signs of hemorrhage. The weight of the animals in this group changed significantly: the maximum drop in weight (approximately by 5.5%) was noticed on the fifth day, but by the 45^{th} day the weight of the dogs had exceeded the original level by more than 5%. /190

In the fifth group, one out of the five dogs died ten days following irradiation. Beginning with the first day, however, the activity of the dog dropped sharply and soon it began to show fluid feces with admixture of mucus. Beginning with the 7^{th} day, we observed complete refusal to eat. The body weight dropped rapidly and despite the early death of the animal, it had fallen by more than 20% by the end of its life. During the last days of life there was a sharp drop in all white blood indices.

In the other four dogs of the fifth group, the disease proceeded much more mildly. Typical diarrhea was observed in only one dog. In two dogs we observed signs of hemorrhage: deterioration and mild hemorrhaging of the gums in one dog, isolated hemorrhaging in the mucous membranes of the gums in another. However, none of the animals showed any hemorrhaging from the nose or oral cavity.

Finally, in the sixth group as in the fifth, one dog out of five died, 18 days after exposure. The clinical picture of radiation sickness for the dogs in this group was quite clearly pronounced. During the first two to three days all five dogs showed a decrease in activity and interest in food. In two dogs,

following the period of improvement from the 12th to the 15th days, activity and interest in food again decreased, especially sharply in the dog which died soon after. In these two dogs, however, with a severe degree of radiation sickness, we observed a typical diarrhea with admixture of blood in the feces and on the 14th through 15th days after irradiation we also observed other signs of hemorrhagic syndrome: deterioration and hemorrhaging of the gums, bloody excreta from the nose. In the surviving dogs on the 19th day of the sickness we also saw numerous hemorrhages in the mucous membranes of the mouth.

The mild symptoms of hemorrhaging of the gums during the period between the 20th and 28th days of the sickness was also observed in one of the dogs which survived. In both dogs with severe radiation sickness, the weight had dropped by 14 - 15% by the 15th day, and in the surviving dog it did not recover even by the 60th day following irradiation, amounting to only 84% of the original level. In the remaining three surviving dogs, the body weight returned practically to the original level by the 30th day. This, in short, was the clinical picture of radiation sickness in the control and shielded dogs. Let us also consider the changes in certain indices of the peripheral blood in these same animals (in the first and sixth groups we studied only the total number of leucocytes).

In all dogs, beginning with the first days after irradiation, /191 we observed a rapid decrease in the total number of leucocytes (Figure 1). In the control animals, the decrease in the number of leucocytes was particularly rapidly progressive and extended until the time of death (up to several hundreds and sometimes several tens of cells per mm^3 of blood). In the protected dogs, there was a much lower level of this index noted in the period from the 7th through the 20th days.

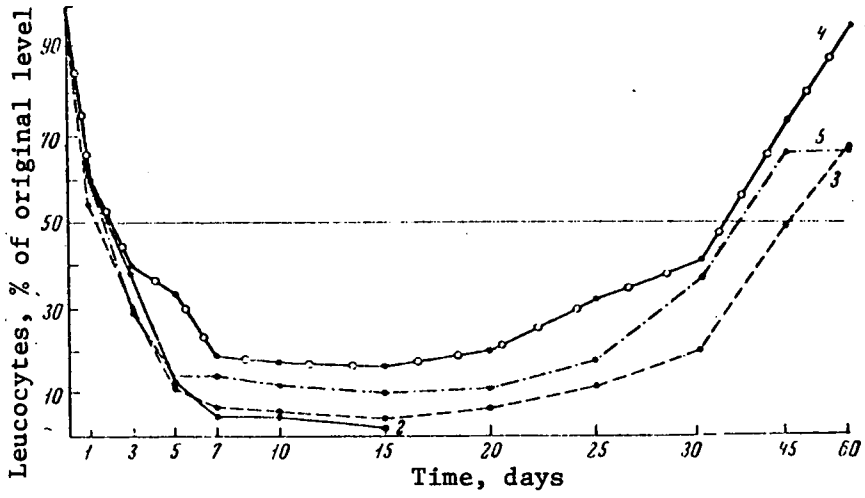

Figure 1. Number of leucocytes in the peripheral blood of dogs at various times following total gamma irradiation at a dose of 600 r.

2 - without shielding; 3 - shielding of the head; 4 - ditto, stomach (residual dose behind the shielding - 150 r); 5 - ditto, stomach (residual dose behind the shielding - 200 r).

The decrease in the number of leucocytes was much more pronounced in the dogs with shielding of the head: in these, on the 15th day the average number of leucocytes fell approximately to 500 cells per mm^3 of blood, which amounted to only 4.2% of the original level. In the animals with shielding of the stomach with blocks that reduced the dose by a factor of 4 (fourth group), the lowest content of leucocytes in the blood was observed also on the 15th day. However, the average number of leucocytes was much higher than in dogs with shielding of the head, and amounted on the average to 2000 cells per mm^3 of blood (16.8% of the original level).

In the fifth and sixth groups, the increase in the total number of leucocytes in the peripheral blood of the dogs occupied

an intermediate position between analogous indices of the other two groups of protected dogs examined above.

By the 30th day none of the groups of protected dogs showed recovery of the total number of leucocytes. The lowest index at this time was found in the third group, 20.3%, while the highest was in the fourth group — 41.2%. On the 60th day, the dogs in the fourth group essentially underwent a total restoration of the total number of leucocytes (up to 95% of the original level), while in the 3rd and 5th groups this was not yet observed. The number of leucocytes in these groups did not reach even 70% of the original level. In the sixth group, on the 30th day the number of leucocytes amounted to only 24% of the original level. Regular observation was not carried out in this group after the 30th day.

It is important to point out that the majority of dogs in the third, fourth, and fifth groups were under observation for more than 60 days, and many of them were subjected to blood tests on the 75th day, while individual animals were studied at still later intervals. An analysis of these far from complete data still makes it possible to conclude that even after 60 days there is a sinusoidal variation of the number of leucocytes in the peripheral blood of irradiated dogs, and in individual groups the number of leucocytes decreases with time to 50 - 60% of the original value.

The analysis of the hemograms of the dogs of the four examined groups (second-fifth) showed that the curve for the change in the number of neutrophils following irradiation corresponds completely to the changes in the total number of leucocytes during the same periods of examination (Figure 2). The lowest figures for the average number of neutrophils by

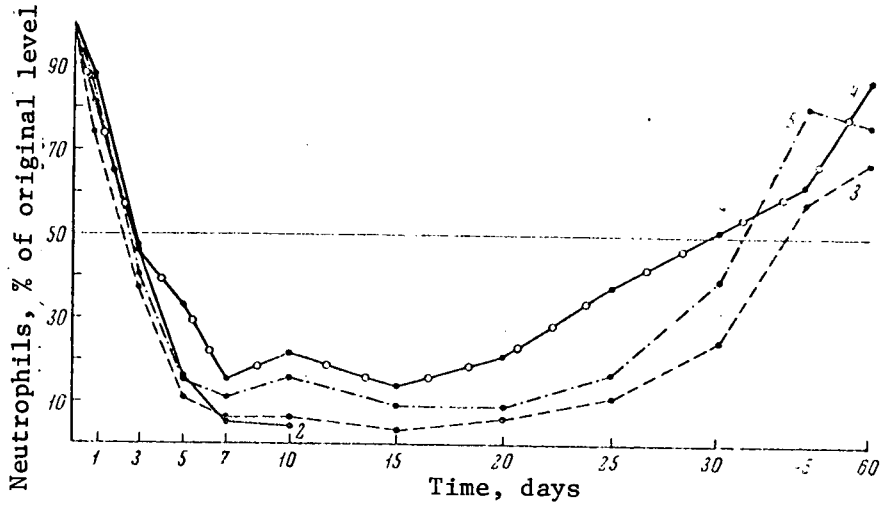

Figure 2. Number of neutrophils in the peripheral blood of dogs at various times following total gamma irradiation at a dose of 600 r.

Symbols the same as Figure 1.

groups are found in the group from the 5^{th} - 7^{th} to 25^{th} days. Among the protected animals at this period of observation, the smallest number of neutrophils was found in the blood of dogs with protection of the head, in which the number of neutrophils on the 15^{th} day dropped to 240-250 cells per mm^3 of blood, which amounted to 3.3% of the original level. In four out of five dogs in this same group, we observed a more pronounced leftwise shift of the hemogram (up to the myelocytes) in the period from the 15^{th} through the 25^{th} days.

In the dogs of the fourth group on the 15^{th} day following irradiation, the average number of neutrophils was about 900 cells per mm^3 of blood (14.1% of the original value). However, they did show a less severe myeloid growth of the marrow, which was confirmed by a more pronounced leftward shift (toward juvenile forms) of the leucocytharic formula during the same periods of observation.

In the dogs of the fifth group the number of neutrophils on the 15^{th} through the 20^{th} days dropped to 620 - 630 cells per mm^3 of blood, which was approximately equal to 9.3 - 9.5% of the original value. The leftwise shift of the neutrophils toward the myelocytes was observed in only one dog of the five that were investigated.

By the 30^{th} day, in all groups there was a noticeable increase in neutrophils in the peripheral blood of the dogs: in the third group almost up to 25%, in the fifth group up to 39.5% and in the fourth group up to 50.8% of the original value. However, on the 60^{th} day there was no complete recovery of this index in any one of the groups of dogs that were irradiated with shielding of individual parts of the body. The highest number of neutrophils at this time was observed (as we would expect) in the dogs of the fourth group — approximately 88% of the original value.

In all the dogs of the fourth investigated group, there was /194
a very rapid drop in the content of lymphocytes in the peripheral blood (Figure 3). In the dogs of the third group, there was a sharply pronounced lymphopenia observed from the 3^{rd} through the 30^{th} days, while during the period from the 3^{rd} through the 20^{th} days the number of lymphocytes was 7.5 - 9.1%.

In dogs with shielding of the stomach, the decrease in the number of lymphocytes and the duration of lymphopenia were less. In dogs of the fifth group, the number of lymphocytes three days after irradiation was approximately equal to 12.5%, while in the dogs of the fourth group it was about 15% of the original value (on the fifth day).

The number of lymphocytes was restored still more slowly than the number of neutrophils. By the 60^{th} day, the dogs with shielding

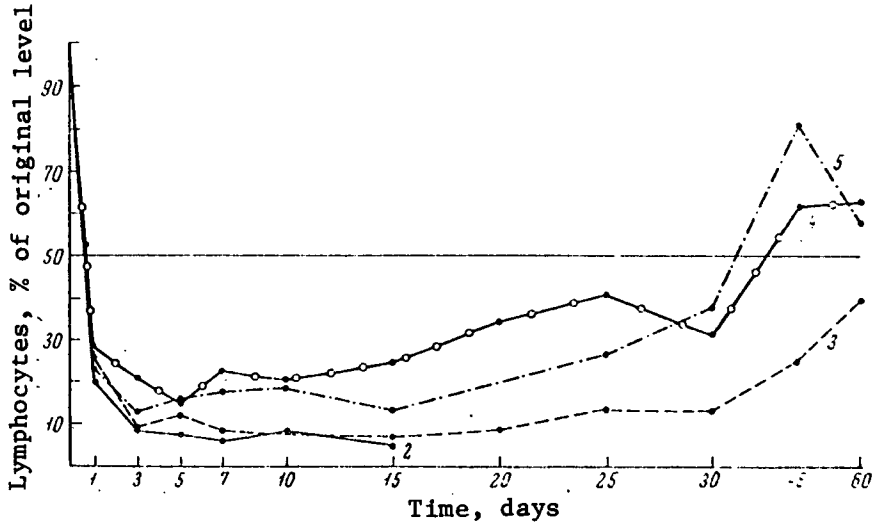

Figure 3. Number of lymphocytes in the peripheral blood of dogs at various times following total gamma irradiation at a dose of 600 r.

Symbols the same as in Figure 1.

of the head had values of only 40% of the original, while the dogs with shielding of the stomach had 55.0 and 62.5% respectively in groups 5 and 4.

The changes in the number of erythrocytes and the quantity of hemoglobin at all periods of investigation were identical in the animals of all groups. A significant drop in the above-mentioned indices took place during the second half of the first month following irradiation. On the 25^{th} through 30^{th} days, the number of erythrocytes in the shielded dogs fell to 55 - 63%, while the quantity of hemoglobin fell to 57 - 80% of the original value. The maximum degree of decrease of these red blood indices was in the dogs with shielding of the head.

By the 60th day, in all of the surviving shielded animals, there was essentially complete restoration both of the number of erythrocytes and the quantity of hemoglobin.

All dogs on the third through the seventh days following irradiation showed a sharp drop in the content of reticulocytes. However, in the shielded animals on the 15th through 20th days, rapid restoration had begun, and by the 25th through 30th days the quantity of reticulocytes had increased to 180 - 270% of the original level. The high content of reticulocytes (2.3 - 2.8 times higher than the original level) in all groups of protected dogs was retained even on the 60th day.

These were the changes in the principal blood indices in all groups of irradiated dogs. A detailed study of all the materials obtained showed that shielding did not prevent damage to the hemopoeitic organs. In all groups of dogs during the first days following irradiation, there was a very rapid drop both in the total number of leucocytes and in the quantity of neutrophils and especially lymphocytes. However, even at these early stages one could see that the damage to hemopoiesis in various groups was not the same.

In dogs with shielding of the head the above-mentioned indices of the white blood during the first two weeks were less pronounced in magnitude than those same indices in the control dogs. However, in those dogs with shielding of the middle section /195 of the stomach by blocks, with the same radiation dose attenuation as with shielding of the head, the total number of leucocytes and the number of neutrophils even on the fifth day — and the quantity of lymphocytes still earlier (from the 3rd day following irradiation) —were much higher than in those dogs which were in

the control group and those that had shielding of the head. In animals with shielding of the stomach with blocks with lesser degree of radiation dose attenuation (by 3 and 2 times instead of 4), the curve showing the changes in the indices of the white blood during the first two weeks following exposure occupied an intermediate position between those same curves for animals with shielding of the head or the stomach by blocks with four-fold attentuation of the irradiation dose.

At still later times, when all the control animals had already died, the nature of the curves reflecting the changes in the indices of the white blood in the protected dogs remained unchanged: a very high level of the indices was observed in the fourth group and the lowest in the third.

The most characteristic and important changes in the red blood indices, as we would expect, began later and were not so clearly pronounced as was the case for the white blood indices. However, the differences in the average number of erythrocytes and the quantities of hemoglobin between the groups of protected dogs at the same periods of investigation were not so clear as was observed in a comparison of the white blood indices.

These are the characteristics of the clinical course of radiation sickness and changes in blood indices in irradiated dogs of all groups. For a more precise and well-founded conclusion regarding the results of the experiment, we have analyzed the clinical symptoms and blood indices at various periods of time following irradiation of the dogs and established the severity of irradiation sickness in all animals, both in the control groups and in the groups with protection of the head and stomach. The data on the acuity of radiation sickness and their outcomes were

examined in close connection with the location of the protective shielding and the degree to which it attenuates the radiation dose — or more precisely, in conjunction with the residual dose behind the shielding. All these data are summed up in Table 2.

We have summarized the results that were obtained in groups 4 - 6, although this slightly impairs the statistics of observations for shielding of the stomach, since ten dogs out of 17 had a residual dose behind the shielding that was higher than for dogs with shielding of the head.

As we can see from Table 2, all of the control dogs developed typical acute radiation sickness of severe degree with a lethal outcome. At the same time, however, with shielding of the head only three dogs out of seven showed radiation sickness of severe degree, which led to the death of these animals. Four dogs survived.

The course of radiation sickness as observed in dogs with /196
shielding of the stomach was still more favorable. Only three dogs out of 17 developed radiation sickness of serious degree, while four developed a moderate degree of severity. However, in the majority of animals with shielding of the stomach (10) radiation sickness proceeded much more mildly, in the form of first-degree severity. With shielding of the stomach, only a total of three dogs died. A particularly high protective effect was observed in animals with protection of the stomach by a shielding that would reduce the radiation dose (as in the group with protection of the head) by a factor of 4. In this group, not a single dog showed typical signs of radiation sickness of the third degree, even in those which died on the 33rd day after exposure.

TABLE 2. DEPENDENCE OF THE SEVERITY OF RADIATION SICKNESS IN DOGS ON THE LOCATION OF PROTECTIVE SHIELDING AND RESIDUAL DOSE BEHIND THEM.

Group	Location of shielding	Degree of dose attenuation by the shielding	Dose behind the shielding, r	Number of dogs		Degree of severity of radiation sickness		
				Total	Survived	III	II	I
1	Without shielding	-	500	7	0	7	-	-
2	Without shielding	-	600	6	0	6	-	-
3	Shielding of the head	4.1	~150	7	4	3	4	-
4	Shielding of the stomach	4.1	~150	7	6	-	1	6
5	Shielding of the stomach	3.0	200	5	4	1	2	2
6	Shielding of the stomach	2.0	300	5	4	2	1	2
4-6	Shielding of the stomach	2-4	from 150 to 300	17	14	3	4	10

The relatively small number of dogs in each group did not allow us to carry out a comparative statistical analysis of the main numerical indices by groups of animals. Nevertheless, based on the composite evaluation of the results of the experiment based on both the mortality factor and the number of dogs with radiation sickness of the same degree of severity in the groups, we were able to draw a number of completely reliable conclusions.

First of all, shielding parts of the body in dogs during /197
total gamma irradiation at a lethal dose is an effective method of increasing their radioresistance, leading to an important attenuation of the severity of radiation sickness in the majority of animals and a decrease in the number of lethal outcomes.

With different doses of total gamma irradiation and equal residual doses behind the shielding, the protective effect of shielding the middle section of the stomach is higher than the effect of shielding the head, with equal masses of protected tissue.

The high protective effect of shielding with a total gamma irradiation of dogs in lethal doses is provided by protecting the tissue of the head or the stomach, whose mass amounts to 10 - 13% of the body weight. To obtain a high protective effect of shielding the stomach under conditions of general gamma irradiation of dogs in lethal doses, it is necessary to ensure a three-fold or four-fold attenuation of the radiation by the material of the protective block. High effectiveness of shielding the stomach in dogs is retained even with a two-fold attenuation of the radiation dose, which is not observed in similar experiments on rats.

The results of the experiment completely supported the basic data that were obtained on rats, and revealed the high effectiveness of shielding in irradiation of larger animals. We have gained the definite impression that protection of portions of the body in dogs during total gamma irradiation at a dose that is in excess of the minimum absolute lethal dose was even more effective than in rats. This conclusion is based on the following data from our experiments.

First of all, shielding the head in dogs had a much greater protective effect than in rats: the survival of the dogs in this case was about 60%, and only 30% for the rats.

Secondly, the high survival rate of the dogs (80 - 85%) with shielding of organs and tissues of the midsection of the stomach, whose mass amounts to 10 - 12% of the mass of the body, was observed not only with a four-fold attenuation of the radiation dose by the protective shielding as was the case in experiments with rats, but also with attenuation of this dose by three and even two times.

In conclusion, we feel it is necessary to say some words about the degree to which the results we obtained in our experiments on dogs agree with the data of other authors.

The effect of the protective action of shielding individual organs and parts of the body, especially the spleen and marrow, in experiments on small laboratory animals has been known for a long time. However, the value of protecting small sections of the body of large animals at lethal radiation doses was not experimentally proven for a long time. There are not many studies in which the effectiveness of shielding parts of dogs' bodies has been studied. They have mainly been published in the last decade.

Among these works, it is interesting to note the studies of /198
a group of authors [5, 6] performed on dogs subjected to the action of hard x-rays, produced by an industrial generator. They found that the dose which would produce the death of half of the irradiated animals (LD_{50}), with shielding of the rear half of the body, was equal to approximately 1775 r, approximately seven times higher than for total irradiation (approximately 250 r). When the front half of the body was shielded, the LD_{50} was equal to approximately 900-950 r, i.e., it was less than four times higher than the LD_{50} for total irradiation. Consequently, in the case of dogs, according to the data of Hansen, Michaelson and Odlund, shielding of the rear half of the body including the region of the stomach provides a much higher protective effect than shielding the front half of the body including the head. It is necessary to note in this connection that the authors of these papers, like the overwhelming majority of other scientists, used shieldings behind which the residual dose did not exceed 1% of the radiation dose. In other words, in their studies, in contrast to our experiment, they provided practically complete protection of the shielded tissues. Nevertheless, regardless of the number of differences in the conditions under which the experiment was performed, the principal finding of the authors is in complete agreement with the data from our studies. Recently, a paper was published written by Yu. G. Grigor'yev et al. [1] which presents the results of a study of the effectiveness of shielding the head and stomach in total irradiation of dogs by protons with an energy of 250 MeV at the minimum absolutely lethal dose. On the basis of their observations, the effectiveness of shielding the head is higher than that of shielding the stomach with an equal mass of protected tissue (15%). All of the dogs survived, regardless of whether the head or stomach was shielded. However, the clinical picture

of radiation sickness and the degree of damage to hemopoiesis in the dogs with shielded heads was significantly less pronounced than in dogs with shielding of the stomach. The results of Grigor'yev et al. differ considerably not only from the results we obtained, but also from the data of the overwhelming majority of other investigators.

The authors explain the effect they obtained first of all by the fact that, when the head is shielded, retention of the centers regulating the principal vitally important functions of the organism is important, although they note that at the comparatively low radiation doses they selected one could scarcely expect organic damage to the brain structure. Secondly, in the opinion of these authors, retention of the most important branch of the pituitary-adrenal system is of considerable importance in shielding of the head. Finally, an important role is played by protection of rather large portions of the active marrow (13 - 17% of its mass), located in the occipital bones, the lower jaw and the upper cervical vertebrae. At the radiation dose selected by the authors, it is the latter factor which plays the principal /19
role in insuring the protective effect of shielding the head.

We feel that in our experiment also the protection of a relatively large mass of active marrow insured comparatively high survival rate of the dogs with shielding of the head, although it was significantly below the survival rate for shielding the same mass of tissue in the vicinity of the stomach.

In our opinion, one of the most important reasons for the significant difference between the basic conclusions drawn in the work of Grigor'yev et al. and our studies is the difference in the magnitude of the radiation doses selected. Unlike Grigor'yev et al., we subjected the dogs to irradiation at a dose considerably

in excess of the minimum absolutely lethal dose. As we know, at high lethal doses of radiation, in conjunction with the outcome of radiation sickness, shielding not only portions of the active marrow, but shielding the intestine as well, is important. This is why shielding the stomach in our experiment was more effective than shielding the head, with equal masses of protected tissue.

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Effect of Shielding the Stomach on the Frequency of Chromosomal Aberrations in the Cells of the Marrow of Guinea Pigs and Rats with Gamma-Irradiation at Doses of 50 - 200 r

M. V. Vasin and B. L. Razgovorov

ABSTRACT. Twelve and 24 hours after irradiation at doses of 40 - 200 r, the authors failed to find any significant differences in the disruption of the chromosomal complex of the marrow cells in experimental (shielded stomach) and control animals with radiation. However, after three days the frequency of chromosomal aberrations in the marrow of the experimental animals had practically returned to the original level and was statistically reliably lower than in the animals that were irradiated without physical protection. The disruption of the chromosomal complex in the experimental animals was also slightly lower than in the animals that were irradiated with half the dose of radiation, which indicates a decrease in the radiation effect by 2 - 2.5 times.

Shielding of portions of the marrow, spleen and intestine leads to a more rapid recovery of organs and tissues in an irradiated organism. F. N. Barakina and M. I. Yanushevskaya [1, 5] observed that shielding the intestine in mice irradiated with a dose of 700 r promotes reparation processes in the marrow of animals which are marked in particular by a more rapid decline in the level of chromosomal aberrations in the marrow cells, beginning on the third day following exposure.

We know that at superlethal radiation doses, the damage to /20
the intestine is the most important factor in the radiation sickness syndrome. Therefore, shielding the region of the

stomach at such radiation doses has been found to be the most effective location of the protective shield [2 - 4]. However, data on the influence of shielding the stomach (intestine) on radiation damage to other organs at relatively small doses of ionizing radiation are missing from the literature available to us. In the present paper, data are presented on the influence of shielding the middle section of the stomach on the frequency of chromosomal aberrations in the nuclei of marrow cells in guinea pigs and rats following gamma-irradiation at doses of 50 - 200 r.

The experiments were performed on 45 male guinea pigs weighing 400 - 500 g and 35 random-bred white female rats weighing 150 - 170 g which were kept under ordinary vivarium conditions on a standard diet. Both species of animals were divided into four groups: I - radiation control; II - radiation control at half dose; III - shielding of the stomach; IV - biological control.

The animals were subjected to general gamma irradiation on the "Khizotron" machine with a Co^{60} source at a dose intensity of 12.5 r/min: the guinea pigs received a dose of 100 r (Groups I and III) and 50 r (Group II), while the rats received a dose of 200 r (Groups I and III) and 100 r (Group II). Shielding of the stomach was carried out using the method described earlier in [3] with the aid of steel blocks placed directly in front of the umbilical line.

Twelve, 24 and 72 hours following exposure, the animals were sacrificed by decapitation. The femoral bones were fixed in a mixture of absolute alcohol and glacial acetic acid mixed in a ratio of 3:1 and stored in 70° alcohol. The cytological investigation of the marrow was performed on "pressed" preparations

with preliminary staining of the material with acetocarmine. Analysis of the marrow cells involved counting the number of normal cells at the stages of anaphase and early telophase, as well as the number of anaphases and telophases with chromosomal disturbances which appeared in the form of typical bridges (dicentric chromatids) and fragments.

The level of chromosomal aberrations (CA) in the cells of the marrow of intact animals (biological control) was as follows: 4.3 for guinea pigs and 2.7% for rats. Gamma radiation in doses of 50 - 200 r produced a reliable increase in the CA level during the first days following irradiation ($p < 0.05$). Thus, in guinea pigs irradiated with a dose of 100 r, after 12 hours the frequency of the CA was increased by a factor of 3, while in rats 24 hours after exposure to a dose of 200 r the level of disruptions increased by more than seven-fold (Table 1, Figures 1 and 2). Later we observed a decrease in the frequency of CA, but even on the third day following exposure (Table 2) the control guinea pigs (100 r) and rats (200 r) showed a level of /2 disruptions in the marrow cells that was reliably higher than in the intact animals ($p < 0.05$).

In groups with shielding of the stomach, the frequency of CA 12 and 24 hours following exposure did not differ significantly from the level of disturbances in the control groups, irradiated with the same doses. However, on the third day following irradiation, the level of the CA in the marrow of the experimental rats and guinea pigs was reliably less than in the control animals ($p < 0.05$).

An analysis of the structural disturbances of the chromosome complex in the marrow cells of the experimental animals showed

TABLE 1. FREQUENCY OF CHROMOSOMAL DISTURBANCES IN THE MARROW CELLS OF GUINEA PIGS AND RATS WITH SHIELDING OF THE ANTERIOR PORTION OF THE BODY DURING THE EARLY STAGES FOLLOWING GAMMA IRRADIATION

Animals	Group	Dose, r	Time of sacrifice following irradiation, hours	Number of animals	Number of cells observed in anaphase and telophase	Anaphases and telophases with chromosomal disturbances	
						Number	% ±m
Guinea pigs	Biological control	-	-	3	1567	67	4.3 ± 0.1
	Radiation control	100	12	3	1707	207	12.1 ± 0.3
	Shielding of the stomach	100	12	3	1711	211	12.4 ± 0.3
	Radiation control	100	24	5	2765	265	9.6 ± 0.5
	Shielding of the stomach	100	24	4	2207	207	9.4 ± 0.2
	Radiation control	50	24	4	2128	128	6.0 ± 0.2
Rats	Biological control	-	-	3	925	25	2.7 ± 0.3
	Radiation control	200	24	3	851	172	20.2 ± 3.7
	Shielding of the stomach	200	24	3	562	106	18.9 ± 2.2
	Radiation control	100	24	3	994	94	9.4 ± 0.7

TABLE 2. FREQUENCY OF CHROMOSOMAL ABERRATIONS IN THE MARROW CELLS OF GUINEA PIGS AND RATS WITH SHIELDING OF THE ANTERIOR PORTION OF THE STOMACH THREE DAYS AFTER GAMMA IRRADIATION.

Animal	Group	Dose, r	Number of animals	Total number of cells examined in ana-telophase	Number of normal ana-telophases
Guinea pigs	Biological control	-	3	1567	1500
	Radiation control	100	4	1828	1700
	Shielding of the stomach	100	4	2099	2000
	Radiation control	50	4	2112	2000
Rats	Biological control	-	3	925	900
	Radiation control	200	5	2645	2500
	Shielding of the stomach	200	5	2383	2300
	Radiation control	100	5	2605	2500

(Continued)

TABLE 2. FREQUENCY OF CHROMOSOMAL ABERRATIONS IN THE MARROW CELLS OF GUINEA PIGS AND RATS WITH SHIELDING OF THE ANTERIOR PORTION OF THE STOMACH THREE DAYS AFTER GAMMA IRRADIATION.

Animal	Group	Anaphases and Telophases						Total number of ana-telo-phases with chromosomal disturbances	
		With chromatid fragments		With chromatid bridges		With chromosomal fragments			
		No.	%	No.	%	No.	%	No.	%
Guinea pigs	Biological control	25	1.6 ±0.2	42	2.7 ± 0.2	0	0	67	4.3 ± 0.1
	Radiation control	39	2.1 ± 0.2	84	4.4 ± 0.3**	5	0.3	128	6.8 ± 0.2**
	Shielding of the stomach	25	1.2 ± 0.2*	72	3.4 ± 0.2*	2	0.1	99	4.7 ± 0.3*
	Radiation control	31	1.5 ± 0.2	75	3.5 ± 0.1**	6	0.3	112	5.3 ± 0.1**
Rats	Biological control	18	1.9 ± 0.1	6	0.6 ± 0.2	1	0.1	25	2.7 ± 0.3
	Radiation control	85	3.2 ± 0.2**	49	1.9 ± 0.1**	11	0.4	145	5.5 ± 0.3**
	Shielding of the stomach	52	2.2 ± 0.3*	26	1.1 ± 0.2	5	0.2	83	3.5 ± 0.1*
	Radiation control	66	2.5 ± 0.3	31	1.2 ± 0.1**	8	0.3	105	4.0 ± 0.1**

(Concluded)

*Between the experimental group and the radiation control at the same dose, $p < 0.05$

**Between the radiation control and the biological control, $p < 0.05$.

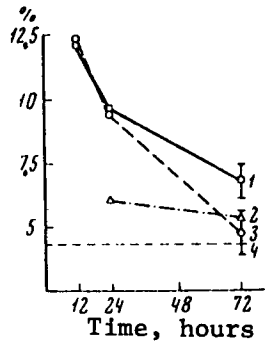

Figure 1. Frequency of chromosomal disturbances in the nuclei of marrow cells of experimental and control guinea pigs following gamma irradiation at doses of 50 - 100 r.

1 - 100 r; 2 - 50 r; 3 - shielding of the stomach plus 100 r; 4 - control.

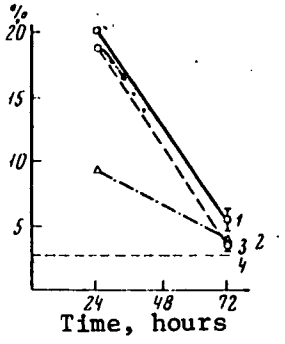

Figure 2. Frequency of chromosomal disturbances in the nuclei of marrow cells of experimental and control rats following gamma irradiation in doses of 100 - 200 r.

1 - 200 r; 2 - 100 r; 3 - shielding of the stomach plus 200 r; 4 - control.

that the decrease in the number of aberrant cells was caused to an equal extent by the elimination of cells with both chromatid fragments and with chromatid bridges ($p < 0.05$).

The CA level in animals with shielding of the stomach on the third day was also slightly less than in the control animals with half the dose of radiation. Thus, for example, in the /20
experimental rats (200 r), the CA frequency at this time amounted to 3.5%, while in the controls which were irradiated at doses of 200 and 100 r the number was 5.5 and 4.0%, respectively.

One of the reasons for radiation death of the cells in the tissues of the animal is the damage by ionizing radiation to the biologically important structural elements of the cells, in particular, the chromosome complex. In the process of

post-radiation recovery, the frequency of chromosomal aberrations in the cells of radiosensitive tissues decreases gradually to the initial level, particularly due to the multiplication of non-lethal damaged cells and the elimination from the cell population of cells with severe disruptions of the chromosomes, usually in the process of cellular division.

The absence of significant differences in the level of aberrant cells in the marrow for protected and controlled animals in the early stages following irradiation (12 and 24 hours) indicates the considerable role played by the direct effect of irradiation in the development of structural disturbances of the chromosome complex. This also indicates that during these times the shielded areas of the tissue of the stomach (intestine) still do not have a marked influence on the radiation damage to the other organs. However, subsequently the portions of the tissue which are shielded during irradiation, apparently by some humoral method, stimulate the reparation processes in the hemopoietic tissue, which frequently takes the form (as studies have shown) of a more rapid decrease in the level of aberrant cells in the cell population.

These experimental data also indicate that the protective /204
effect of shielding the stomach at low damaging doses of radiation (50 - 200 r) is expressed to approximately the same degree as in irradiation at lethal doses at which a decrease in the severity of radiation damage by approximately 2 - 2.5 times is observed [4].

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Aminotransferases of the Blood Serum of Dogs Following Total Exposure to Gamma Rays with the Stomach or Head Shielded

B. I. Davydov and Ye. Ye. Simonov

ABSTRACT. In the serum of the blood of dogs subjected to a single irradiation with Co^{60} at a dose of 600 r (2.9 r/min) with protection of the head or stomach, the authors investigated the activity of glutaminoaspartic (GAST) and glutaminoalanic (GALT) aminotransferase. Hyperfermentemia was observed from 40 to 90 days. Exposure to radial acceleration (to the point of disruption of cardiac activity) causes uniform hyperfermentemia in intact dogs and in those irradiated with the stomach shielded and no changes in GALT and GAST activities in dogs with shielding of the head. The authors explain these differences by the changes in the activity of aminotransferase, and the rate and volume of the reparative processes of different shielding conditions.

According to current concepts, the activity of aminotransferases in the blood serum depends on the output of these enzymes from the tissues. The direct cause of the liberation of aminotransferases from the tissue is either necrosis of the cells or an increase in the permeability of the cell membranes,

which is due to disruption of the oxidation and oxidation phosphorylization processes and a decrease in the formation of macroergic compounds [1, 4 - 7, 11, 12].

Numerous studies have shown that there is a positive correlation between the increase of activity of aminotransferases and the intensity of pathological processes in the tissues, which makes it possible to utilize the changes in the enzyme activity as an indicator of the depth and volume of the damage. On the other hand, the level of hyperfermentemia may serve as an index of the reparative processes aimed at the elimination of foci of injury. All of this provides a basis for assuming that a study of the dynamics of the activity of aminotransferases in the serum of the blood may also be valuable for evaluating the influence on the organism of a harmful factor such as ionizing radiation. In the present paper, we present the results of determining the activity of aminotransferases — glutamicoaspartic (GAST) and glutamicoalanic (GALT) in the blood serum of irradiated animals with various parts of the body partially shielded against ionizing radiation. /20

The experiments were performed on mongrel male dogs weighing approximately 12 kilograms which were subjected to a single unilateral gamma irradiation with Co^{60} at a dose of 600 r (2.9 r/min). A detailed description of the shielding method is presented in the paper by B. L. Razgovorov [8].

B. L. Razgovorov and N. A. Gaydamakin established that with shielding of the anterior portion of the stomach the following were protected: half or most of the spleen, the liver, 4 - 4.5 vertebrae, portions of 2 - 3 ribs, the right kidney and half or 2/3 of the left kidney, both adrenals, a portion of the loop of the small intestine (up to 1 meter or more), the anterior

portion of the large intestine, a large part of the stomach, etc. When the head was shielded, 2 - 3 cervical vertebrae were protected as well. The shielded mass of tissue in both cases amounts to 12 - 15% of the weight of the entire body. The dose behind the shielding is attenuated 3 - 4 times, i.e., the residual dose amounts to 150 - 200 r. The survival rate for animals with shielding of the stomach reached 83%, and 57% when the head was shielded [8].

The activity of animotransferases was determined in serum obtained from the venous blood of experimental dogs using the method of Umbright in the modification of T. S. Paskhina. For the study, we collected 0.5 ml of serum, diluted twice with distilled water, with retention of the assumed ratios of residual ingredients of the reaction media. The activity of the enzymes was expressed in micrograms of pyroracemic acid (PRA).

As we can see from Table 1, irradiation of dogs with the stomach shielded, practically speaking, is not accompanied by a decrease in the activity of GAST in the blood serum at the early stages (during the first 8 days) or radiation sickness, while the activity of GALT during the first days is noticeably decreased, by 30% in comparison to the controls. At later observation stages (40 - 90 days) the activity of both enzymes goes considerably beyond the control level. The maximum increase in the activity of GAST is noticed in 40 days; after 60 and 90 days, the activity of the enzyme decreases, but not down to the control levels. The maximum rise in the activity of GALT was recorded 60 days after radiation exposure; after 90 days, the GALT activity varied slightly relative to the preceding period of study.

According to the data in the literature [14], in the acute form of radiation sickness there is a sharp decrease in the

TABLE 1. CHANGE IN ACTIVITY OF ASPARTIC AND ALANINE TRANSFERASE (GAST AND GALT) IN THE BLOOD SERUM OF DOGS IRRADIATED AT A DOSE OF 600 r WITH THE STOMACH OR HEAD SHIELDED (150 r BEHIND THE SHIELDING)*

Group	No. of dogs	Time after irradiation, days	GAST		GALT	
			PRA, μg	% of control	PRA, μg	% of control
Without shielding	6	—	75 ±4,2	100	64±8,7	100
Shielding of the stomach	1	1	65	86	45	70
	2	3	71 (70—72)	95 (93—96)	60 (46—75)	94 (72—117)
	2	5	78 (74—82)	104 (98—109)	65 (64—66)	101 (100—103)
	2	8	77 (76—78)	102 (101—104)	53 (52—55)	83 (81—86)
	2	40	157 (150—165)	210 (200—220)	90 (75—105)	140 (117—164)
	2	60	119 (65—173)	158 (86—230)	129 (74—184)	202 (106—287)
	5	90	97±4,0	125 ±5,3	112±10,0	175±15,6
Shielding of the head	3	90	103±11,5	137±15,3	87±3,6	136 ±5,6

*Commas represent decimal points.

activity of GAST and GALT in the blood serum during the first days (1 - 7 days) following exposure. Subsequently, there is a recovery of the activity of these enzymes to the initial level and even above it. The initial decrease in the activity involves inhibition of synthesis of enzymatic proteins, which is indicated in particular by the unequivocal nature of the changes in the enzymatic activity in a number of tissues [9]. It must be said, however, that in the liver the activity of GAST and GALT is not reduced; on the contrary, according to the data of I. V. Savitskiy and V. V. Tsybul'skiy [9], it increases markedly in comparison with the control, literally from the first day following exposure. The mechanism and biological significance of this effect are not completely understood. /20

In the case which we studied, the general dynamics of activity of GAST and GALT in the blood serum coincided with that observed in the experiments of Varga et al. [14], but differed in a quantitative respect. These differences included, on the one hand, a shorter and less pronounced decrease in the activity of aminotransferases in the early stage of radiation sickness and, on the other hand, a later (although more marked) subsequent increase in the activity of GAST and GALT. The absence of a sharp drop in activity of aminotransferases during the first days following exposure, which ceases during the subsequent increase in activity of these enzymes, must obviously be evaluated as a significant reduction in the damaging effect of radiation with stomach shielding.

During the period of increased activity of GAST and GALT in the blood serum in the experimental animals, we observed almost complete recovery of the clinical, hematological [8] and certain biochemical indices, for example, the composition of the protein fractions (Yu. K. Syzrantsev and B. I. Davydov). This fact

leads us to assume that at this stage of post-radiation damage there is no complete normalization of the physiological (especially certain biochemical) processes — as is the case in the restoration of the "fine" structure of the organs and tissues — so that conditions are created which favor the retention of the high permeability of the cell membranes and elution of intracellular enzymes from the tissues. Judging by our data, this condition is maintained for the entire observation period, which has a total length of up to three months.

All that we have said relative to the changes in the activity of aminotransferases in experiments on the influence of radiation with shielding of a portion of the stomach may be extended to the results obtained in determining the activity of GAST and GALT in experiments with irradiation when the head is protected (see Table 1). In this case, the activity of GAST and GALT in the blood serum of experimental animals remains at a high level until the end of the third month. A comparison of the data from the experiments with irradiation and shielding of the stomach and head will show that the degree of hyperfermentemia 3 months after exposure in both cases is almost the same. In addition, it is apparent that it must be greater when the head is shielded, since the organs of the abdominal cavity are not protected at this time and consequently are damaged to a greater degree than in the animals which have the stomach protected. It is possible that here a more severe initial inhibition of GAST and GALT synthesis is observed in the damaged organs, so that the restoration of their biosynthesis, like the course of the reparative processes in the tissues, remains somewhat retarded. /2

The labile (although compensated under ordinary conditions) restorative processes in the irradiated animals may be expressed more completely under the influence of some kind of provocative

factor which leads to acute tissue hypoxia. As such an influence, we selected transverse overloads, one of whose principal pathogenic mechanisms is oxygen starvation of a circulatory and hypoxic nature [2, 3].

Some of the dogs which had survived radiation sickness were exposed to the action of radial accelerations. All of the animals used in such experiments survived 90 days after exposure. The overloads were imposed on a centrifuge with a 4.25 meter arm, and the action of the overloads was directed from the chest to the back. The EKG was recorded for the animals with the first, second and third leads, as well as the respiration frequency. The increase in the overloads was performed at the rate of 0.2 units per second until signs of cardiac activity disruption were noted (increase in the RR interval by a factor of 2), after which the centrifuge was slowed down at the same rate.

Transversely directed overloads in experimental and control dogs produced almost identical changes in the cardiac activity, characteristic for this type of influence [2, 3]. When the overloads were increased, we usually observed a shift in the ST interval relative to the isoline and an increase in the T spike. A slowing down of the frequency of cardiac contractions was accompanied by the development of extrasystole (Figure 1). A reduction of the R spike was observed somewhat more frequently in the irradiated dogs than in the controls. As we know, the most informative aspect of the EKG is the RR interval and as an average value, the frequency of cardiac contractions. In the control animals, under the influence of overloads, the frequency of cardiac contractions increased and reached a maximum at 8 - 10 units, after which there was a decrease in the frequency of the pulse to the original level, 20 - 22 units. Then there was a sharp bradycardia, which served as a signal for slowing down

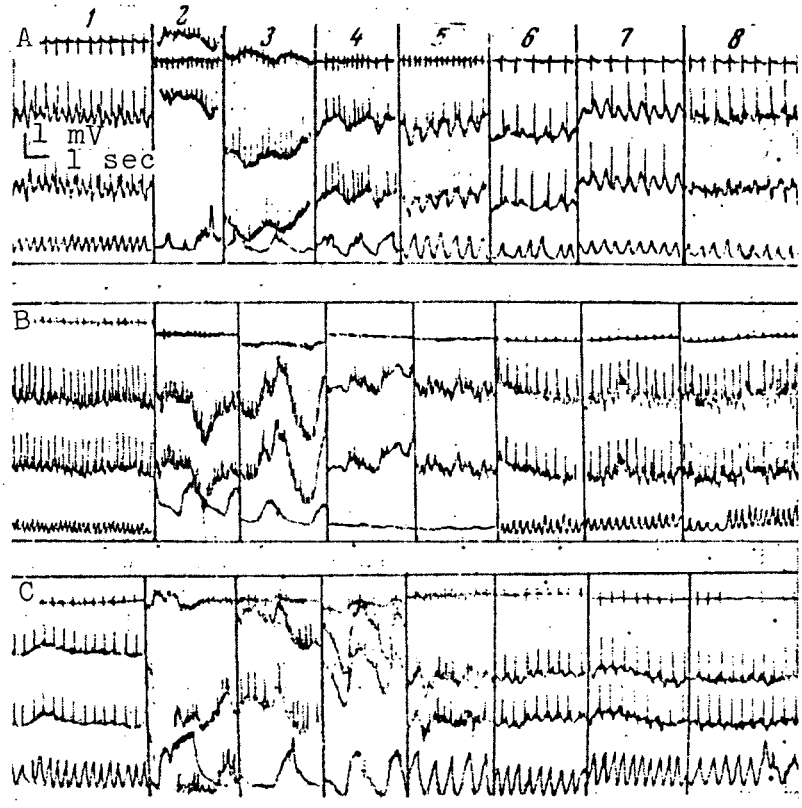

Figure 1. EKG for control dog 24 (A) and animals irradiated with shielding of the stomach (Dog 6, B) and head (Dog 15, C), subjected to overloads:

1 — background; 2 and 3 — fifth and fifteenth minutes of increase; 4 and 5 — fifteenth and fifth minutes of decrease; 6, 7, 8 — immediately, fifth and fifteenth minutes after the centrifuge is stopped

(Table 2, Figure 2). The values of the maximum tolerable overloads are given in Table 4. With braking to 10 - 4 units, tachycardia in the control dogs was evidenced almost as much as during the period of "rise". Five minutes following the descent, the /211

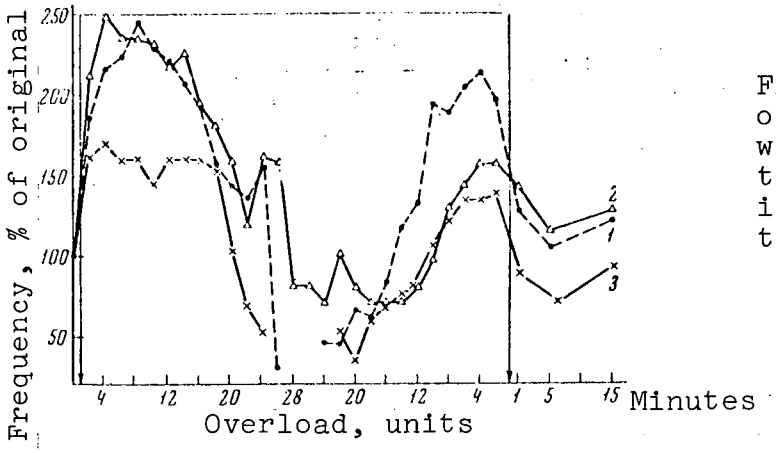

Figure 2. Frequency of cardiac contractions with overload in control (1) and in animals irradiated with protection of the stomach (2) and head (3)

pulse rate was within the limits of the original values. The dynamics of the changes in the pulse frequency during increased overloads in animals with the stomach area protected was the same as in the control dogs. However, during braking, tachycardia was less pronounced (see Figure 2). The frequency of the cardiac contractions (in absolute units) during overloads in Dog 15 with the head protected differed slightly from the control animals (see Table 2). In Dog 4, the original value of the pulse frequency was higher (210 beats per minute) than in the other animals. During overloads, the absolute values of the pulse frequency in this animal reached the same values as for the other dogs. However, in relative values the changes in this index were less pronounced, which had an effect on the average value for this group (Curve 3, Figure 2). The high degree of excitability of cardiac activity in this animal apparently was related

TABLE 2. FREQUENCY OF CARDIAC CONTRACTIONS IN DOGS DURING CENTRIFUGING 90 DAYS AFTER GAMMA IRRADIATION AT A DOSE OF 600 r

Group	Dog	Original value	Overload, units													Following rotation		
			4	8	12	16	20	24	28	24	20	16	12	8	4	1	5	15
Control	22	150	300	330	260	330	300	210		60	90	90	150	240	300	120	130	150
	23	120	300	330	300	180	70				70	70	150	260	330	280	200	220
	24	130	270	330	330	270	210				90	180	240	270	250	120	100	130
	M	134	290	330	300	260	193	210		60	83	113	180	257	293	173	143	166
	%	100	216	246	223	194	143	140		40	62	84	134	192	218	129	106	124
Shielding of the stomach	7	120	300	240	240	240	180	100		70	90	60	90	150	180	150	120	130
	6	120	300	300	300	300	240	300	100	100	180	180	150	150	150	180	160	190
	8	160	330	360	330	300	240				60	50	90	180	240	90	150	170
	20	90	300	270	210	120	120				60	60	60	150	150	50	100	140
	M	122	307	290	270	240	194	200	100	85	98	88	98	160	180	175	132	160
	%	100	250	238	220	196	160	167	83	71	80	72	80	131	148	144	118	131
Shielding of the head	4	210	330	330	300	300	240	90			60	90	130	270	270	150	120	200
	15	240	270	240	240	240	120				120	150	150	160	210	160	130	130
	M	175	300	285	285	285	180	90			80	120	140	215	240	155	125	165
	%	100	170	162	162	162	103	43			46	69	80	123	137	89	71	94

to a more pronounced residual symptom of radiation injury. This dog had the lowest value for the number of leucocytes (4.1 thousand), erythrocytes (7.1 million) and hemoglobin (13.8 g %). During the increase and decrease of the overload, the changes in the respiration frequency were approximately the same for all groups (Table 3). However, there were differences following the overload. While the control dogs had a frequency of respiratory movements that reached original values by the 5th minute following rotation, the animals with shielding of the stomach had values that were 30% higher (mainly due to Dog No. 7), while with protection of the head, on the other hand, they were lower by 20%. At the 15th minute, the frequency of respiration in the control dogs remained within the limits of the original values. During this period, in two experimental animals, we observed a tendency toward a decrease in the frequency of respiratory movements: by 50% (Dog 7) and 88% (Dog 4) below the original level, respectively, in the group with protection of the stomach and the head (Table 3).

After 2 hours, 1 and 7 days following rotation, we determined the activity of glutamate-asparate and glutamate-alanine — transferases, cholinesterase, serotonin, ceruloplasmin and bradykinin in the serum (G. A. Chernov). In addition, we performed a general analysis of the blood (N. V. Arnet, S. N. Komarova).

In this paper, we shall present only the results of studies of aminotransferases. The results of the determinations are listed in Table 4.

As we can see from this table, the influence of overloads led to a significant and rapid increase in the activity of GAST in non-irradiated and irradiated dogs with the stomach area

TABLE 3. FREQUENCY OF RESPIRATION (IN MINUTES) FOR DOGS UNDER THE INFLUENCE OF OVERLOADS 90 DAYS AFTER GAMMA IRRADIATION AT A DOSE OF 600 r WITH SHIELDING OF THE STOMACH OR HEAD

Group	Dog	Original value	Overload, units													Following rotation, minutes		
			4	8	12	16	20	24	28	24	20	16	12	8	4	1	5	15
Control	22	310	60	50	50	50	40	40		40	40	40	60	80	80	220	320	300
	23	130	50	50	40	40	30				30	10	10	20	80	130	210	240
	24	190	80	60	40	50	50				40	30	50	80	70	60	130	120
	M	210	63	53	43	47	40	40		40	36	27	40	60	76	136	220	220
	%	100	30	25	20	22	19	13		13	17	13	19	29	36	65	105	105
Shielding of the stomach	7	80	80	40	30	30	30	40		10	—	10	10	20	100	230	320	40
	6	310	60	20	40	40	50	50	20	20	—	—	—	—	60	170	200	230
	8	320	90	70	50	50	30				20	20	30	70	100	270	360	330
	20	160	70	40	40	50	50				30	30	20	40	55	220	250	110
	M	215	70	42	40	42	40	45	20	15	25	20	20	43	77	222	282	177
	%	100	33	20	18	20	18	23	6	8	10	11	11	24	36	103	130	83
Shielding of the head	4	250	130	120	80	90	60	10		10	20	—	—	80	130	180	200	30
	15	180	50	30	40	30	30				30	30	50	60	90	110	140	150
	M	215	90	75	60	60	45	10		10	25	30	50	70	105	145	170	90
	%	100	42	35	39	25	28	4		4	11	17	28	32	49	67	79	42

TABLE 4. CHANGE IN ACTIVITY (IN MICROGRAMS OF PYRORACEMIC ACID), ASPARTIC-AMINOTRANSFERASE (GAST) AND ALANINE-AMINOTRANSFERASE (GALT) OF THE BLOOD SERUM OF DOGS SUBJECTED TO OVERLOADS 90 DAYS FOLLOWING GAMMA IRRADIATION WITH THE STOMACH OR HEAD SHIELDED (150 r BEHIND THE SHIELDING)*

Group	Dog	GAST				GALT				Maximum tolerable overload, units
		Original value	Following overload			Original value	Following overload			
			2 hrs.	1 day	7 days		2 hrs	1 day	7 days	
Control	24	105	114	—	—	116	100	—	—	23
	22	97	141	108	103	87	109	87	103	26
	23	77	290	244	64	59	147	112	80	21
	M	93,0	181,6	176,0	83,5	87,3	118,6	99,5	91,5	23,3
	%	100	195	201	96	100	135	135	125	—
Shielding of the stomach	6	105	220	—	175	132	185	—	150	29
	8	85	165	—	115	100	140	—	—	22
	20	80	126	—	80	150	145	—	110	22
	7	72	173	192	64	71	120	115	108	27
	M	85,5	171,0	192,0	108,5	113,2	147,5	115,0	122,6	27,5
	%	100	200	266	126	100	130	161	107	—
Shielding of the head	15	122	122	—	87	85	108	70	70	21
	4	80	104	132	52	56	43	61	47	25
	M	101,0	113,0	132,0	69,3	70,5	75,5	61,0	58,5	23,0
	%	100	112	165	68	100	106	87	83	—

*Commas represent decimal points.

protected, while in the latter this defect was significantly higher in magnitude than in the non-irradiated animals. The GALT activity in the animals of both groups increased to a lesser degree than the activity of GAST (Figure 3).

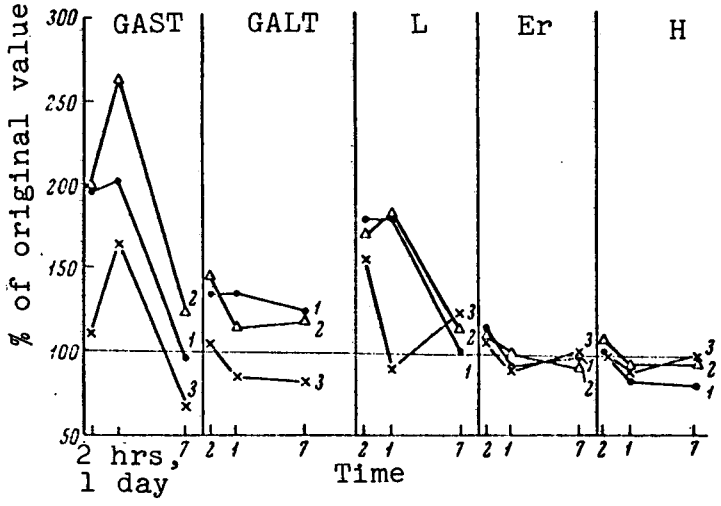

Figure 3. Change in activity of aspartic-aminotransferase (GAST), alanine-aminotransferase (GALT), quantity of leucocytes (L), erythrocytes (Er) and hemoglobin (H) in control (1) and irradiated dogs with shielding of the stomach (2) and head (3) 2 hours, 1 and 7 days following exposure to overload

The influence of accelerations on dogs irradiated with protection of the head was expressed in a slight increase in the activity of GAST during the period from the first two hours to one day, and a subsequent decrease not only in comparison to the original value but relative to the level of activity in intact animals prior to centrifuging. However, the increase in GALT activity was noticeable in only 1 dog, while in another there was even a slight decrease. /213

The results of these observations indicate that centrifuging leads to similar effects in non-irradiated and irradiated dogs with the stomach area protected. The absence of evident damage to the organs at the selected load of exposure makes it possible to explain the hyperfermentemia by the development of hypoxic states which arise as a result of hemodynamic disturbances. The condition of hypoxia is always accompanied by disruption of the oxidation processes and oxidation phosphorylization [10, 15], which is considered one of the possible causes of an increase in cellular permeability and escape of intracellular enzymes from the tissues into the circulatory system [13]. In animals which have been exposed to irradiation with shielding of the head, hyperfermentemia was slightly higher in terms of intensity than in experiments with centrifuging of intact dogs. If we consider the approximately equal intensity of the influence of radial accelerations in both cases and consequently the approximately equal degree of hypoxia which develops under these conditions, we must assume that the more pronounced increase in the activity of GAST and GALT in the irradiated animals has to do with the qualitative "inferiority"[1] of the structural elements of the tissues, both those that are retained as well as those that are formed instead of being destroyed. One of the frequent signs of this "inferiority" of the cells may be the increased permeability of the cellular membranes, as indicated by the higher level of hyperfermentemia in the dogs that were irradiated with protection of the stomach following centrifuging. In the quantitative respect, however, the volume of the reparative processes at this stage apparently approaches the state of the tissues and

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[1]We are using the word "inferiority" here to designate primarily the functional immaturity of young cell forms, which are less stable to the hypoxic factor.

organs in intact dogs. While prior to rotation the number of leucocytes in these dogs was decreased (Table 5), the potential /215
possibilities of reparative processes apparently resembled those of healthy animals.

We know that following the action of overloads there is a leukocytosis of a redistributive nature. In our experiments, the nature and severity of the leukocytic reaction in response to overloads in animals with protection of the stomach was the same as in the control group (see Figure 3). However, it must be pointed out that leukocytosis in the experimental group would have been much greater if we had not taken Animal 7 into account, in which the leukocytic reaction was absent for 2 hours. The severity of the leukocytic reaction in animals with stomach protection is almost the same as for the control dogs and it indicates a similar degree of reactivity of this system in response to overloads.

From this standpoint, the slight increase in activity of GAST with a subsequent fall below the original level, with an absence of clear changes in the activity of GALT in the blood serum of dogs in which the head was shielded during exposure, may be viewed as an index of both the qualitative and quantitative insufficiency of reparative processes in the damaged tissues, which in composition and state is still very far from normal. Leukocytosis was less pronounced in the group with the head protected following overloads, especially in Dog. 4. As we mentioned earlier, this animal differed from the others in other respects as well. Following the action of the overloads, the reaction of the erythrocytes in both the control and experimental dogs was nearly the same. Two hours following rotation, there was a slight increase in the number of erythrocytes and the hemoglobin content and there was a decrease in the

TABLE 5. QUANTITY OF BLOOD CELLS, HEMOGLOBIN AND SERUM VOLUME IN DOGS AFTER OVERLOAD 900 DAYS FOLLOWING GAMMA IRRADIATION AT A DOSE OF 600 r WITH PROTECTION OF THE HEAD OR STOMACH***

Group	Dog	Erythrocytes, millions				Hemoglobin, %				Leucocytes, thousands				Serum, ml		
		Original level	After overload			Original level	After overload			Original level	After overload			Original level	After overload	
			2 hrs.	1 day	7 days		2 hrs.	1 day	7 days		2 hrs.	1 day	7 days		2 hrs.	1 day
Control	22	7,7	7,8	6,6	7,5	15,8	14,6	13,8	13,0	14,2	21,2	17,0	10,9	0,35	0,30	0,35
	23	7,6	9,2	6,7	8,6	16,2	18,4	13,2	14,0	10,1	21,8	29,0	13,7	0,35	0,15	0,30
	24	8,8	11,6	9,7	—	14,2	15,0	13,8	—	11,5	21,3	18,4	—	0,40	0,35	0,40
	M	8,0	9,5	7,7	8,0	15,4	16,0	13,6	13,5	11,9	21,4	21,5	12,3	0,37	0,27	0,35
	%**	100	118	96	100	100	104	88	88	100	180	180	103	100	73	96
Shielding of the stomach	7	9,0	10,2	10,0	7,8	16,4	17,4	16,8	16,8	8,5	8,5	23,0	10,8	0,25	0,20	0,30
	6	8,0	9,6	8,1	8,4	15,0	18,0	15,8	16,0	6,4	15,2	13,3	8,4	0,30	0,15	0,25
	8	8,3	11,3	9,6	8,8	16,0	19,4	16,8	15,8	9,6	18,7	11,8	6,5	0,35	0,25	0,40
	20	8,6	8,1	7,2	7,6	16,4	16,0	13,8	14,8	4,6	7,4	4,9	8,5	0,35	0,35	0,30
	M	8,5	9,8	8,7	8,1	15,9	17,7	15,8	15,8	7,2	12,4	13,2	8,5	0,31	0,24	0,31
	%*	100	115	102	95	100	111	99	99	100	172	183	118	100	76	100
	%**	106	122	119	101	103	115	103	103	60	104	111	71	85	64	85
Shielding of the head	4	7,7	7,8	6,7	7,3	13,8	14,0	12,4	14,5	4,1	5,5	4,8	6,9	0,35	0,15	0,30
	15	8,7	10,3	8,1	9,3	17,2	19,3	16,0	16,4	5,9	10,1	4,2	5,7	0,45	0,25	0,30
	M	8,2	9,0	7,4	8,3	15,5	16,1	14,2	15,4	5,0	7,8	4,5	6,3	0,40	0,20	0,30
	%*	100	110	90	101	100	104	92	99	100	156	90	126	100	50	75
	%**	102	112	93	104	101	104	92	100	42	65	93	53	110	55	82

*% of original value. **% of original value for control.

***Commas represent decimal points.

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volume of serum (see Table 5). Hence, centrifuging is a functional test which makes it possible to determine the reserve capacities of the organism and its systems under conditions of increased stress. The results of these tests indicate that the reserve capacities were less in the animal of the given group, in comparison with dogs that were subjected to irradiation with protection of a portion of the stomach.

The data which we obtained indicate that single general irradiation of animals at a dose of 600 r with shielding of the stomach or head leads to the development of a stable hyperfermentemia (increase in activity of GAST and GALT) which is observed in the time interval between 40 - 90 days. The presence of hyperfermentemia during the period of clinical recovery may be viewed as an index of insufficient reparative processes indicating the possibility of prolonged retention of latent disturbances in metabolism and fine structure in the externally undamaged tissues and organs. Centrifuging of intact and irradiated dogs makes it possible to determine that the reparative processes in animals with shielding of the head is pronounced to a much lesser degree than in animals with shielding in the region of the stomach.

This study also demonstrates the possibility of using the /2
aminotransferase activity in the blood serum as a test characterizing not only the intensity of radiation damage, but also the state of the restorative processes in the irradiated organism.

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Pathomorphological Changes in the Organs of White Rats Following Irradiation by Protons with an Energy of 120 MeV and the Role or Partial Shielding in the Attenuation of Radiation Damage

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ABSTRACT. Rats were irradiated with protons having an energy of 120 MeV at a dose of 640 rads with partial shielding of the stomach. A pathomorphological investigation was carried out on the spleen, testes, stomach, intestine, myocardium, central and peripheral nervous system, and retina. In the irradiated animals we found destructive changes typical for radiation sickness, not only in the radiosensitive organs, but also in the so-called radioresistant ones (myocardium, nervous system). Local protection of the stomach reduced the depth and severity of changes in the organs, especially the ones directly covered by the shielding.

The present paper is devoted to a study of the effect of protons with energies of 120 MeV on the internal organs of animals with total radiation and with partial shielding of a portion of the stomach.

In these experiments, we used 90 male rats with an average weight of 275 g. Irradiation was performed with a collimated beam of protons at the Joint Institute of Nuclear Research at Dubna, using the synchrocyclotron there. The intensity of the /218
dose was 60 $\pm$ 10 rads per minute, and the absorbed dose was 640 rads. In half the irradiated animals, the middle segment of the stomach was shielded (4 cm wide) during the action of the

radiation by means of strips of plastic 15 cm thick. The dose behind the shielding was 5 - 8%. The weight of the shielded segment of the trunk was 20% of the weight of the animal. The rats were killed by ether vapor 1, 3, 7, 15, 30 and 60 days after irradiation (6 animals each time from each of the series under comparison).

Sections of the organs were processed by a number of generally employed histological and histochemical methods and the new neurohistological method of S. G. Kul'kin.

The observations showed that the irradiated animals showed inhibition of growth. A portion of the animals died during various periods: one on the 3^{rd}, 3 on the 10^{th}, and 1 on the 14^{th} day. In those animals which were irradiated with shielding of the stomach, there were no deaths, and the signs of diarrhea in them were less pronounced. The weight of the rats at all periods was practically the same as in the control animals.

When the animals irradiated with protons were autopsied on the 3^{rd} and 7^{th} days, we found hemorrhages in the small and large intestines and in the wall of the stomach. Beginning with the 3^{rd} day and during all subsequent periods, there were non-uniform plethoras and hemorrhages in the pulmonary tissue. In the animals which were killed, we found hemorrhages in the lungs which were extensive and numerous, and in two animals there were hemorrhages in the myocardium, while two others showed them in the wall of the stomach.

We did not observe any large macroscopic changes in the shielded animals. The plethoras and hemorrhages in the lungs,

small and scattered, were observed only in 2 animals which were killed on the 7th day.

Analysis shows that the changes in the relative weight of the spleen and thymus gland were of the same nature. One and 3 days later, these indices were statistically reliably less; after 7 days we observed a tendency toward recovery, but recovery was complete only on the 15th day.

At subsequent intervals, the weight coefficient of the spleen was higher than in the controls, but the weight coefficient of the thymus gland remained at the level of the controls or even increased in some animals.

The nature of the change in the relative weight of the male gonads was different. We recorded a reliable decrease in the weight coefficient of this organ only on the 30th day; by the 60th day there was a further decrease in this index. In protected rats, the changes in the weight coefficients of the spleen and thymus glands were much less evident that in the non-shielded ones. However, the weight coefficient of the testes of the irradiated rats and in those protected by a shielding changed in the same fashion. /219

In a histological examination, all of the organs were found to contain marked changes in the vessels: venous and capillary plethoras, perivascular edemas, erythrocyte extravasation*, hemorrhages in the lungs, the wall of the stomach and intestine, myocardium and tissue of the testes as well as in the choroid of the eye.

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* Editor's Note: This is a discharge of blood from a vessel into the tissues.

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In the spleen after 1, 3 and 7 days we observed marked damage to the follicles and foci of myelopoiesis in the pulp (Figure 1). At these times, there was a sharp increase in the pulp of the number of granulocytes with segmented nuclei, which appeared in their hypersegmentized forms. The erythroblasts disappeared from the foci of myelopoiesis after 1 day, and the megakaryocytes by the 7th day. Regeneration was still only slightly evident on the 7th day. At this time, only 1 animal out of 6 had pulp in the spleen in which small accumulations of erythroblasts could be found. Considerable recovery was achieved on the 15th day: foci of myelopoiesis in the pulp were of larger size, contained excessive amounts of hemocytoblasts and all forms of hemopoietic cells - erythroblasts, myeloblasts, juvenile and segmented-nuclear granulocytes, megakaryocytes. However, the follicles were reduced, with a blurred outline, and contained much more lymphoblasts and prolymphocytes than usual. By the 60th day, the volume and cellular composition of the follicles was abnormal and the myeloid hemorrhaging in the pulp had been repaired.

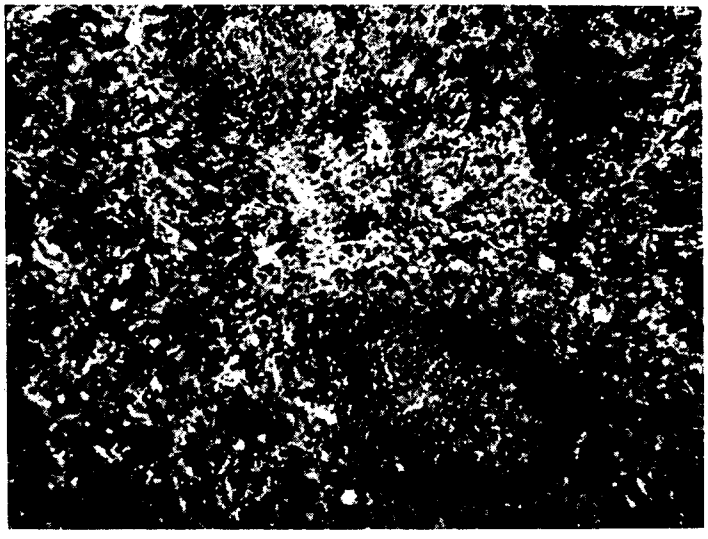

Figure 1. Spleen 24 hours after total irradiation. Shrunken follicle containing almost no lymphocytes. Small accumulations of basophilic reticular cells in the devastated pulp.

Hematoxylin-eosin. Magnification 115 x, objective 16.

In the protected rats, the damage to the spleen was markedly less evident (Figure 1, 2). In many follicles during the first days following irradiation there was retention of centers of multiplication, and lymphocytes were observed. With considerable damage to the foci of myelopoiesis, they retained a small quantity of erythroblasts and megakaryocytes. Clear signs of recovery were already evident by the third day, when the follicles began to accumulate lymphoblasts and prolymphocytes and there were erythroblasts and megakaryocytes at the foci of myelopoiesis. Regeneration of foci of myelopoiesis in the protected animals had mainly terminated by the 15th day, and recovery of the volume and cell composition of the follicles — by the 30th day.

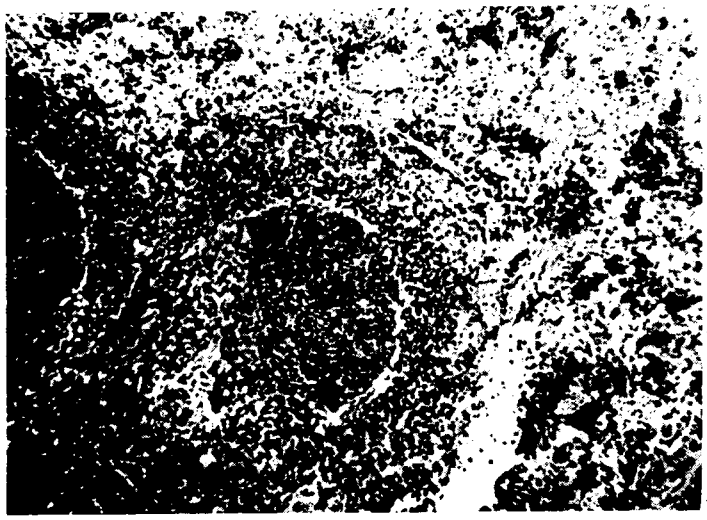

Figure 2. Spleen 24 hours following irradiation with shielding of the stomach. Decrease in follicles less pronounced, follicles contain centers of multiplication and contain lymphocytes. Small foci of myelopoiesis are visible in the pulp.

Hematoxylin-eosin. Magnification 115 x, objective 16.

In the testes, considerable changes took place in the epithelium of the seminal vesicles. The average number of vesicles with spermatogonia in the controls was 86 $\pm$ 7%. Only 24 hours following irradiation, their percentage fell to 74 $\pm$ 3%; on the 3rd day it was 69 $\pm$ 9% and on the 7th day — only 64 $\pm$ 6%. A still more marked impoverishment of the

vesicles with respect to spermatogonia occurred on the 15th and 30th days (45 ± 8% and 43 ± 15%, respectively). Sixty days later, the vesicles lacked almost all forms of reproductive cells, with the exception of a small number of spermatogonia (7 ± 1%). The number of vesicles with spermatocytes in the control was 90 ± 8%. One and 3 days after exposure to protons, there were only 70; on the 7th — 52 ± 4; on the 15th — 58 ± 7; and on the 30th day — 30 ± 6%.

However, similar changes were observed in respect to the /22
number of vesicles containing spermatids and spermatozoids. In addition, the number of damaged vesicles and vesicles containing dystrophically altered cells increased progressively, amounting in 1 day to 13 ± 2, in 3 days to 27 ± 4, in 7 days to ± 4, in 15 days to 29 ± 4, in 30 days to 49 ± 5 and on the 60th day to 93 ± 2%.

In animals which were protected by a shielding, the number of vesicles with individual forms of reproductive cells was higher at all times than in the unprotected animals, although this difference was not always reliable. The statistically significant differences were observed only on the 7th day, on the basis of the number of the vesicles containing spermatazoids and spermatids, and on the 15th and 60th days — with respect to the number of vesicles containing spermatagonia.

In the mucous membrane of the stomach in irradiated rats, beginning with the first few days and subsequently up to 15 days, we observed increasing dystrophic and destructive changes in the gastric epithelium. The lumina of the fundal glands were dilated, overfilled with mucus, and there was vacuolization of the cytoplasm, with swelling of the nuclei in some cells and pyknosis in others; the surface epithelium was desquamated in

places. Especially pronounced destructive changes were observed on the 7th day following exposure. Reactions to acid sulfomycins in the secretory granules of the main cells of the fundal glands, which are clearly evident in the mucous membrane of the stomach of the control rats, were suppressed for 15 days. At the same time, there were initial symptoms of recovery of the mucous membrane. In the layer itself there was accumulation of lymphocytes, and figures of mitosis appeared among the transitional cells. On the 30th day we observed almost complete recovery of the morphological structure of the secretory apparatus of the stomach; the surface epithelium had recovered and the shapes of the fundal glands were again tubular.

In animals which had been subjected to irradiation with partial shielding, the dystrophic and destructive changes in the cells of the superficial and glandular epithelium of the mucous membrane of the stomach were less clearly pronounced than in animals which had been irradiated without shielding of the stomach. On the 7th and 15th days, in the vicinity of the gastric fundus, undamaged fundal glands were still evident and dystrophic changes in the cells developed only on the 3rd day. Reactions to acid sulphomycins in the cells of the fundal glands were suppressed to a lesser degree and were more evident at all stages than in unprotected rats.

In studying the duodenum of irradiated rats after 24 hours to 3 days, we observed increasing edema in the layer of the mucous membrane itself as well as in the submucous layer. The villi were shortened, and in a number of cases had been subject to destruction. Frequently the villi, arranged in rows, were compressed and had a common epithelial coating. In the crypts there were large amounts of cellular detritus and a number of /221
scyphiform cells, overfilled with mucous, and enlarged.

After 7 days one could observe signs of regeneration, whose intensity increased in subsequent periods, and the size of the villi was restored.

The mitotic index (MI) of the cells in the crypts of the mucous membrane of the duodenum in the control animals amounted to 64.5 ± 0.7%. Twenty-four hours after exposure, the MI was 40.8 ± 2% ($p < 0.001$). Simultaneously there was also a decrease in the average content of cells in the crypt (61.6 ± 1.7 versus 93.0 ± 0.6 in the control, $p < 0.001$). Inhibition of mitotic activity was accompanied by an increase in the content of aberrant mitoses: it became 2.5 times greater than in the non-irradiated rats (22.1 ± 1.7 instead of 8.9 ± 0.7%). After 3 days, the MI reached the level of the controls, but the average number of cells in the crypts remained low ($p < 0.001$), while the content of aberrant mitoses increased reliably. After 7 days, the mitotic activity of the cells in the crypts, in comparison with the controls, increased and amounted to 80.2 ± 1.8%, which naturally led to an increase in the average number of cells in the crypts (up to 166.1 ± 0.8). The percentage of aberrant mitoses continued to remain high (14.7 ± 1.1%).

On the 15th day, the MI and the average number of cells per crypt had increased still further (93.6 ± 1.0%, 164.1 ± 1.5 cells), and the percentile content of aberrant mitoses was also high (17.3 ± 0.7). After 30 days, the average number of cells per crypt had decreased to normal, while the MI and the number of chromosomal failures was higher ($p < 0.01$). After 60 days, the mucous membrane of the intestine was again characterized by a large number of cells which were related on the average to one crypt; the MI was slightly smaller than in the preceding period, but was still higher (76.3 ± 2.5%), and the number of

abberant mitoses (13.1 ± 0.5%). The differences were reliable.

Shielding of the stomach, i.e., direct protection of the irradiated segment of the intestine, led to a marked reduction in the structural and cytological changes in its mucous membrane. In the protected rats, in comparison with those that were not protected, edema of the mucous membrane of the duodenum was less noticeable and was observed during only one period, 1 day after irradiation. The villi were subjected to less changes, and we saw fewer disruptions of their structure as well as desquamation of the cells of the epithelium. We did not observe any accumulation of epithelial cells in the crypts. The drop in the mitotic index relative to the controls was less pronounced than in the unprotected animals; the increase in the mitotic index (in comparison with the ordinary conditions) which was observed on the 7^{th} day and in subsequent periods also was not as significant as in the unprotected rats. The same was true for the average number of cells associated with each crypt. /222
A decrease in the number of cells in the crypts which was observed in the non-protected animals after one day was practically absent in the shielded rats; the restoration of their numbers took place more rapidly. The average content of cells in the crypts on the 7^{th} and 15^{th} days approached the ordinary level, and increased only on the 30^{th} and 60^{th} days.

In the heart, the muscle fibers during the first days lost transverse striation during the initial days, and the myocardium showed development of swollen fibers which were homogenized. The background reaction to free amine groups was slightly decreased, and in some fibers there was a marked increase in the NH_2 groups. There was a small amount of fuchsinophilic fibers. As a rule, the nuclei of the muscle fibers were large, and exceeded the size

of the nuclei of the muscle cells of the myocardium of the control animals by 1.5 - 2.5 times. In addition, there were reduced and pyknotic nuclei with basophily of the karyoplasm and a pronounced enlargement of the chromatin structures. On the third day, there was an increase in the number of fibers with fuchsinophilic sarcoplasm, giving a marked positive reaction to NH_2 - SH groups. Fuchsinophilia, gradually increasing, reached its maximum on the 7^{th} through 15^{th} days. At these times, there were very many fuchsinophilic fibers in the wall of the right ventricle, as well as in the subepicardial and subendocardial sections of the left ventricle and in the region of the apex. /22
Several gradations of fuchsinophilia can be seen: from a mild pink color of individual disks of myofibrilae and intercalary disks to intensive red or reddish orange coloring of homogeneous muscle fibers. The reaction with ninhydrin revealed that, together with the large number of fibers containing many amine groups, there were fibers with a markedly reduced reaction. A similar mosaic appearance was observed with the development of sulfhydryl groups. Under luminescence microscopy, many of the fibers that showed coarse fuchsinophilia when treated with acridine orange had a bright green color. In some fibers, we found dust-like deposits of calcium and sudanophilic granules.

On the 30^{th} day, the surviving animals showed a decrease in the dystrophic processes in the myocardium. However, at this time there were fibers that lacked transverse striation; many muscle cells, when treated according to Selye and Mallory, showed a change in tinctorial properties and contained an increased amount of amine and sulfhydryl groups and calcium. After 60 days, the muscle fibers had a clear transverse striation, and some of them were hypertrophic. /22

In the shielded rats, the changes in the structure of the myocardium had the same nature as those described above, but they were less pronounced than in irradiation without protection of the stomach. Attenuation of the intensity of the background reaction to the NH_2 group was less noticeable, although the focal increase in this reaction in individual muscle fibers was evident. We also noticed a more moderate fuchsinophilia of the muscle fibers. Sclerotic changes in the myocardium were nearly absent during the latter stages.

Studies of the brain performed on the 7^{th}, 15^{th} and 60^{th} days following irradiation revealed that in the cerebral cortex of the brain after 7 days, breakup of chromatophilic substance of many cells predominated in the 5^{th} layer, with the rare occurrence of slightly pronounced peripheral tigrolysis. After 15 and 30 days, the changes in the cortex had increased: in addition to normally strained neurons, there were also cells with a reduced Nissl reaction, as well as hyperchromic cells and those with signs of peripheral lysis of chromatophilic substance, vacuolization and swelling of the cytoplasm. On the 30^{th} and 60^{th} days, in the upper and lower layers of the cortex one can see here and there wrinkled cells with swelling or hyperchromic nuclei.

The same sort of pathology (but more pronounced) is evident in the neuronal elements of the Ammon horn* and medulla oblongata. After 7 days, the principal mass of cells shows signs of swelling and lysis of the cytoplasm, pyknosis, karyolysis and cytolysis, and at later stages there are even signs of chromatolysis. The neuroglial reaction is also reduced. In addition, there is also a number of slightly altered cells. The most pronounced

* Editor's Note: This is the hippocampus.

changes of this kind in the medulla oblongata were found 60 days after irradiation.

Shielding of the stomach reduced the degree of tygrolysis and hyperchromotosis of the neurons in various parts of the brain. The protective effect of shielding was observed primarily in the latter stages, beginning at 15 days, and primarily in the columnar formations.

In the intramural nervous system of the bladder, prostrate gland, seminal vesicles, testes and also in the nerve elements of the muscles of the pelvic fundus and the gastrocnemius muscle in the early stages following irradiation, there were considerable reactive (and in later stages, destructive) changes.

Twenty-four hours later, in the transversely striated muscles of the pelvic fundus, the thick pulpy fibers had swelled; in the preterminal motor thrombocytes infiltrations of neuroplasm had occurred, while the farthermost portions of the motor endings, arranged on externally unchanged cushions of motor thrombocytes remained intact. In the ganglia of the spermatic plexus we found cell-shadows, against which background one could sharply make out the outlines of varicose nerve fibers. In the urinary bladder, we found dichromy of the nerve fibers and varicosis of individual afferent conductors. However, the overwhelming majority of the receptor apparatus and fibers forming them had an ordinary appearance. After 3 days, there was marked evidence of varicoses of nerve fibers of the pelvic and spermatic plexuses. There was production of droplets of myelin and breakdown of medullary fibers. The pericellular apparatus became markedly swollen. The hydropic dystrophy of the gangliose cells proceeded similarly to the fine- and large-droplet vacuolization of the neuroplasm (Figure 3). In the

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Figure 3. Pelvic plexus on the third day following total irradiation. Large-droplet vacuolization of neuron. Method of Kul'kin. Magnification: objective 20, ocular 10.

intramural plexuses we observed many hyperchromic non-medullary fibers and irritated afferent conductors, especially in the muscular wall of the urinary bladder.

The maximum changes in the nerve elements of the pelvic organs were observed 15 days following exposure. The synapses were swollen and reached tremendous size, up to 7 - 12 microns, and extended considerably into the perikaryon of one or more neurons. Such terminal sections, being in contact with the bodies of several neurons, were obviously able to carry out a compensatory function, since in other pericellulars a portion of the presynaptic fiber endings had degenerated. We observed vacuolization, pericellular edema and wrinkling of the neurons of the pelvic and spermatic plexuses, increasing (relative to the control) the number of double and polynucleolate cells. Fragmentation involved primarily the /226 medullary leads of the intercellular plexuses of the extramural ganglia, the intramural plexuses of the urinary bladder and prostate gland as well as the efferent fibers of the transversely striated muscles. In the testes, individual nerve bundles consisted only of varicose fibers, whose number was increased on the whole (Figure 4). There was also damage to the vascular nerve plexuses of the pelvic organs. Thus, the disturbance to innervation was combined with vascular disturbances that

Figure 4. Pelvic plexus 15 days following total irradiation. Breakdown of pulp fiber. Bil'shovskiy-Gross. Magnification: objective 20, ocular 10.

complicated the radiation damage of these organs. In the lymphoid tissue, the most damage was done to the terminals of the sensitive nerve endings.

By the 30th day, in the nervous system of the pelvic organs, signs of residual damage remained, and there was a breakup of nerve elements. By the 60th day there was mainly normalization of the morphology of the pericellular apparatus, synapses, efferent and afferent endings in the nervous plexuses of the internal organs and lymphoid tissue. Periaxonal edema in the nerve plexuses disappeared; the perineural sheaths had the usual diameter and were in close contact with the blood vessels.

In rats which were subjected to irradiation with shielding of the middle portion of the stomach, the severity and extent of the damage to the nerve elements of the pelvic organs were less. In this case we observed primarily reactive changes, especially in the first few days; however, the destructive changes that were observed in the non-protected rats developed much more rarely and only at later stages (by the 30th and 60th days). On the 60th day, the pelvic and spermatic ganglia showed individual degenerative changes in the neurons and suppression of the cell-satellite reaction.

A comparison of the damage to the nerve system of the pelvic organs and the nerve elements in areas more remote from the shielding, i.e., the muscles of the rear extremities of the irradiated rats, showed that in these cases the nature and dynamics of the structural changes showed no important differences. The stages of development of the pathological process involved the same periods. Protection of the stomach by a shielding placed in front of the umbilical line was accompanied in both areas by an important decrease in the reaction of nerve structures to direct radiation influence.

In the retina of the eye after 1 and 3 days, among the nerve cells with ordinary location of Nissl substance we found neurons with signs of tigrolysis, focused or central. Sometimes the tigroid accumulated in the periphery of the cells in the form of large lumps. Sometimes the changes led to an extreme accumulation of cells with enlargement and hyperchromy of their nuclei, which sometimes swelled so much that one had the impression that cells were being pushed out of them. After 3 days, it was possible to see among the neurons those whose nuclei contained two or more nucleoli.

More severe reactive and dystrophic disturbances developed /227
on the 7^{th} and 15^{th} days following irradiation. The pathological process involved a larger number of nerve cells than in the preceding periods. The nuclei were frequently wrinkled and acutely basophilic. In individual neurons of the gangliose layer, we could see deep dystrophic changes: vacuolization of the cytoplasm, fragmentation of the nuclei and death of some cells. The dystrophic changes in the form of disruption of the nuclear structure and the ectopy of the nucleoli were also found in the cells of the internal nuclear layer. On the 15^{th} day, there was a breakup of the photoreceptive layer and death

of the rods and cones. However, during this period we also observed neurons without any sign of damage. Pronounced edema of all layers of the retina was seen.

On the 30^{th} and especially on the 60^{th} day following irradiation, the ability of the cells of the retina to form lumps of tigroid in the neuroplasm returned. However, even on the 60^{th} day, this process had not returned completely. At this time, neurons with signs of central tigrolysis were found.

In rats which had been irradiated under conditions of partial shielding of the stomach, the changes in the retina were similar in character to those in the unshielded animals, but the severity was less and during the latter stages the appearance of giant cells was prevented.

In the surface epithelium of the surface of the stomach and the mucous membrane of the duodenum as well as in the myocardium and the gangliose layer of the retina of the eyeball, /228 we measured the volume of the cell nuclei. Karyometric studies revealed that during the initial period there was a swelling of individual nuclei and pyknosis of others. At later stages, on the 15^{th} and 30^{th} days, some large nuclei appeared which were saturated with chromatin, and the development of pyknosis by the nuclei was observed rarely. In protected rats, all of these changes in the volume of the nuclei were much less pronounced at all stages.

Hence, with total single irradiation with protons at an energy of 120 MeV and a dose of 600 rads ($LD_{30/30}$), the rats developed a typical picture of radiation sickness with development of diarrhea, hemorrhagic symptoms, characteristic loss of body weight and weight coefficients of the spleen, thymus gland

and testes.

The spleen showed a marked destruction of the pulp and follicles, disappearance of lymphocytes and cells with myeloid hemopoiesis. Definite signs of recovery of the structure of the organ were noticed only beginning with the 15th day, and by the 16th day complete recovery was not yet evident: the follicles remained reduced, had no definite contours, and contained a reduced quantity of lymphocytes.

In the testes, all reproductive cells disappeared from the epithelium of the vesicles by the 60th day and we did not observe any signs of recovery of spermatogenesis at this time. In the mucous membrane of the stomach, during the first days following exposure and after 15 days, we observed increasing dystrophic and destructive changes in the glandular surface epithelium. Recovery of normal structure of the mucous membrane of the stomach was noticed on the 30th day.

In the duodenum, in addition to the dystrophic and destructive changes in the villi and epithelium of the crypts, during the first few days we observed a suppression of mitotic activity of the cells of the crypts and a decrease in their average number per crypt. Among the cells lining the crypts, the number of aberrant mitoses increased by a factor of more than 2. The signs of regeneration of the mucous membrane of the intestine were observed comparatively early, by the 7th day. Recovery of the morphological picture of the duodenum was essentially complete by the 30th day.

In the myocardium during the first days following irradiation, edema of the muscle fibers was observed with loss of transverse striation, suppression of the background reaction to free amine

and sulfhydryl groups and reinforcement of fuchsinophilla of muscle fibers. There were also signs of lipophanerosis and deposition of calcium in the fibers. All of these changes in the myocardium must be viewed not only as dystrophic but also as destructive, accompanying the denaturing of the myosin, which also shows up in luminescence microscopy. At later stages, the changes in the myocardium were not clearly evident: there was moderate sclerosis of the interstitial tissue in the walls of the vessels, primarily in the form of productive cell reactions.

Significant reactive dystrophic and partially destructive changes showed up in the brain, especially in the subcortical formations and in the truncus. Swelling and hypochromy of the cytoplasm and nuclei of the neurons were frequently accompanied (especially in the later stages) by the development among them of hyperchromic nuclei with signs of pericellular edema and fragmentation. /229

In the peripheral nervous system, the changes took on a more coarse nature than in the brain. In the nerve elements of the wall of the urinary bladder, seminal vesicles, prostate gland, pelvic lymph nodes and also in the nerve elements of the skeletal muscles of the pelvic fundus and the gastrocnemius muscle, even at early stages of radiation sickness, there were significant reactive and destructive changes that led to the breakdown and fragmentation of medullary and partly non-medullary nerve fibers, preterminals, sensors and motor receptors.

There were significant reactive and destructive changes in the neurons of the retina, especially in the gangliose layer and the photoreceptive elements. Individual neurons showed tigrolysis as well as swollen and hypochromic nuclei, and on the 30^{th} and 60^{th} days there was vacuolization and fragmentation

of the nuclei.

In those animals which were irradiated with 120 MeV protons at a dose of 640 rads with shielding of the middle part of the stomach, the changes described above were also observed, but they were markedly decreased. This was evidenced by the fact that there were no deaths among the protected animals, diarrhea was encountered more rarely, as well as being less severe and brief in duration. Hemorrhages were rarer in the lungs, and were not observed at all in other organs. Recovery of body weight and weight coefficients of the spleen and thymus gland took place at earlier times.

In a histological examination of the spleen, destruction of follicles and medulla was less evident, recovery of lympho- and myelopoieses began and ended much earlier and was more complete.

It should be pointed out that the protective effect of the shielding on the thymus gland, subjected to the direct action of protons, judging by the weight coefficients is manifested to a lesser degree than in the spleen, which was protected by the shielding during irradiation. We can assume that the radiation damage to the spleen developed under these irradiation conditions, not because of the residual dose which was not absorbed by the shielding, but rather because of the remote effect of the radiation, through toxic products of radiolysis of water, amino acids, lipids and other substances, i.e., through radiotoxins formed in the organs subjected to the direct action of protons. In addition, the decrease in the damage to the spleen and other organs protected by the shielding reduced the direct action of radiation on the thymus gland.

The same result of reducing the direct action of radiation /230
was evident in the histological examination of the other organs. The decrease in the dystrophic and destructive changes in the spleen, the mucous membrane of the stomach and the duodenum which were covered by the shielding and evidently in the liver and a very important endocrine gland - the adrenals, reduced the severity of radiation damage to the myocardium, the central and peripheral nervous system and the retina. Recovery of radiation damage of the protected organs also began at much earlier times and was more complete. Less pronounced protection with shielding of the stomach was evident in the testes. However, here too, when the vesicles were examined for reproductive cells of various kinds, there was only a slight decrease in the suppression of spermatogenesis.

A reduction of the damage to the central and peripheral nervous system apparently plays a role which is not critical in the change (under the influence of partial shielding) in the reaction of the organism to radiation effects, inasmuch as we know that the nervous system is of considerable importance in the regulation of normal functions and trophicity of organs and systems. Protection of the adrenals by a shielding using corticosteroid hormones may obviously promote normalization of the endocrinotrophic regulation of the reparative processes in the organs damaged by ionizing radiation.

One of the indicators of improvement of tissue trophicity under the influence of partial shielding was the attenuation of changes in the nuclear proteins. This was indicated by a less severe variation of the nuclei volumes of the cells in many organs, evidenced by a karyometric study.

Conclusions

1. General single irradiation with protons having an energy of 120 MeV at a dose of 640 rads produced typical radiation sickness in rats, with characteristic damage to organs and tissues. Pronounced dystrophic and destructive changes occurred not only in the radiosensitive but also in the so-called radioresistant organs, such as the myocardium and the nervous system.

2. The universal reaction to the damaging effect of ionizing radiation consists of diverse changes in the volumes of nuclei of cells in organs with both high and low proliferative capacity.

3. Shielding of the stomach, reducing the dose in the protected area to 5 to 7% of the initial value, clearly changes the reaction of the rat organism to the action of protons, reducing the radiation damage to the organs. There is a marked decrease in the depth and severity of the changes in the spleen, gastrointestinal tract and also in the organs which are not protected by the shielding — in the central and peripheral nervous system, myocardium and retina.

4. The mechanisms which attenuate the radiation damage to the /231 organs and tissues with use of local protection of the animal include a decrease in the damage to the hemopoietic (spleen and marrow, vertebrae and ribs) and endocrine (adrenals) organs. Hence, there is acceleration of hemopoiesis reparation and an improvement in the endocrinotrophic regulation of the reparative processes in the organs and tissues of the irradiated organism.

N73-20077

Significance of Radiosensitivity of Individual Parts of the Body in the Development of Radiation Pathology

N. A. Gaydamakin

ABSTRACT. Male rats were subjected to total gamma-neutron irradiation at a dose of 300 rads (neutron contribution about 90%) with the head and stomach protected by a shielding. The test data included mortality, frequency of diarrhea, the nature of the pathomorphological changes in the organs and the degree of recovery of the reproductive function in the irradiated males. In the rats subjected to total gamma-neutron radiation the characteristic feature of radiation sickness was earlier damage to the intestine. Placing the screen over the stomach is a more effective means of antiradiation protection than over the region of the head.

The problem with which the current paper deals is the study of the characteristics of pathomorphological changes in the organs of rats which were subjected to gamma neutron irradiation with shielding of the head and the area of the stomach.

In the experiments, we used 130 random-bred white male rats with an average weight of about 200 grams. The animals were distributed among four series: 40 rats were subjected to radiation alone, 36 rats had the head shielded during irradiation, and 39 had the stomach shielded. Fifteen rats served as a biological control.

The animals were subjected to a single general gamma-neutron irradiation at a dose of 300 rads with a predominant contribution of neutrons (approximately 90%) to the absorbed dose (dose intensity = 12 rads per minute). For shielding, we used blocks made of plastic 15 cm thick, and sheet lead 1 cm thick, which decreased the dose of neutrons by 4 and 6, and the dose of gamma radiation — by two and three times. In locating the shielding in the vicinity of the head, in conjunction with the fashion in which the animals were held down, the block protected the entire head, cervical vertebrae, as well as the upper extremities to the level of the brachial girdle. The weight of the protected tissue amounted to about 15% of the weight of the rat. When the stomach was shielded, the block was 4 cm wide, mounted in front of the umbilical line and protected the area of the liver, stomach, upper part of the pancreas, the adrenals with the upper poles of the kidneys, four vertebrae, and 4 - 6 ribs, which together with the soft tissues made up about 20% of the body weight of the rat.

For pathomorphological study, the animals were killed with ether vapor six hours, 3, 7, 15 and 30 days, 3, 5 and 9 months

after irradiation, with 3 - 4 rats being killed each time. The /232
organ sections were processed with generally accepted histological, as well as some histochemical and neurohistological methods. In the intestine, we determined the mitotic activity of the crypt cells. The state of spermatogenesis was evaluated by the method of Fogg and Cowing [12].

During the first two days, the irradiated rats in all three series did not differ from the intact ones in terms of their external appearance and behavior. Beginning with the third day, the animals ate less food and their motor activity decreased noticeably as well. Beginning with the 7^{th} day and on subsequent days, the animals had an ordinary appearance. Beginning with the 30^{th} day, all of the irradiated animals showed loss of fur; after 7 to 9 months the rats showed foci of epilation, while some animals had lost weight by this time.

Some of the rats died at different times after irradiation. Deaths were first noticed after 5 days among the unprotected rats, on the 8^{th} day with protection of the head and on the 10^{th} day with protection of the stomach. Diarrhea was observed in only a few rats and began in the irradiated animals (without protection) and with protection of the head on the third day, while with protection of the stomach it began on the fourth day and lasted for no more than 2 - 4 days.

In the animals sacrificed on the 3^{rd} and 7^{th} days, the spleen and thymus gland were reduced in size. In some rats,

the small intestine had edematous walls, expanded lumina and contained a foamy yellow liquid. When the head (and especially the stomach) were protected, changes in the intestine were less pronounced than in the unprotected rats, and were encountered more rarely. In the lungs, beginning on the 3rd and 30th days, many animals showed hermorrhaging of various degrees, individual and numerous. In the table, we have listed the data which were obtained from a macroscopic examination and which characterize the degree of radiation damage to the animals in each series.

Fifteen days following irradiation the testes were shrunken, which was especially noticeable in subsequent days. Three and a half months later, the weight of the testes was reliably less /234 than in the controls: in the unprotected rats it amounted to 820 ± 125 mg, with protection of the head it was 883 ± 105 mg, and with protection of the stomach it was 916 ± 150 mg. In the control rats, the weight of the testes was 3233 ± 160 mg.

In the histological examination of the animals subjected to irradiation, the latter show inhibition of hemopoiesis in the marrow and spleen with a breakdown of hemopoietic cells and subsequent significant destruction of these organs. Recovery of the structure of the hemopoietic organs, regardless of the comparatively early start of regeneration (7 - 15 days), was incomplete, especially in the spleen; 9 months following irradiation, it was possible to note signs of radiation damage in this organ.

In the initial part of the small intestine, marked changes were evident in the mucous membrane, as indicated by the death and desquamation of a considerable part of the epithelium

TABLE 1

INFLUENCE OF IRRADIATION CONDITIONS ON DEGREE OF RADIATION DAMAGE IN RATS

Group	Mortality, %		No. of rats with:		
	By 30th day	By 100th day	Diarrhea on 4th day	Intestinal damage (up to 7 days)	Pulmonary hemorrhage (up to 30 days)
Without protection	30	40	15(34)	6(6)	7(16)
Protection of head	20	40	9(30)	4(6)	6(14)
Protection of stomach	11	11	2(33)	2(6)	5(12)

Note: The number of rats is shown in parentheses.

covering the villi, breakdown of the cells of the lymphoid accumulations and certain prismatic cells in the bottom of the crypts, and a brief suppression of mitotic activity in the crypts. Subsequent increased regeneration of the cells in the crypts did not allow total recovery of the structure of the mucous membrane, which at all periods showed signs of atrophy with acceleration and thickening of the villi, vacuolar dystrophy of the cells of the epithelial coating (Figure 1), and after 30 days a second decrease in the number of mitoses in the crypts. The mitotic index of the cells of the intestine crypts in intact animals amounted to (1:1000):42.6 $\pm$ 3.5; 6 hours after irradiation, there were no mitoses. After 3 days there were 68.5 $\pm$ 4.8; after 7 days, there were 74.3 $\pm$ 4; after 15 days there were 85.3 $\pm$ 5.7; and after 30 days there were 20.0 $\pm$ 4.2. Damage to the testes was characterized by disappearance of the cells of the germinative epithelium: the spermatagonia disappeared on the 15th day, the spermatocytes and

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spermatids on the 30th day, and the spermatazoids disappeared later on (Figure 2). Regeneration of spermatogenesis was not observed, and the reproductive function remained suppressed in the males for 9 months.

In the brain, the most significant changes of a destructive nature occurred during the first hours following neutron exposure. Six hours after exposure, the olfactory bulbs of the brain around the narrow band, which are covered by ependymal cells, showed large accumulations of sharply altered cells with pyknotic nuclei and cell fragments. A portion of the cell detritus was phagocytized by the so-called granular spheres with characteristic /233 placement of absorbed granules in the cytoplasm in the form of rosettes around the nuclei. This cellular detritus appeared also in the olfactory and frontal portions (Figure 3). In the sagittal sections, accumulations of products of cellular breakdown could be seen in the form of highly evident "paths", running from the olfactory bulbs to the anterior horns of lateral ventricles (Figure 4). At the point where the anterior horns of the lateral ventricles have the shape of narrow fissures, the nuclear fragments surrounded them on all sides. As a measure of the expansion of the anterior horn cavities,

Figure 1. Jejunum. Three days after irradiation. Shortening and exposure of villi. Hematoxylin-eosin, magnification 90 x, ob. 8.

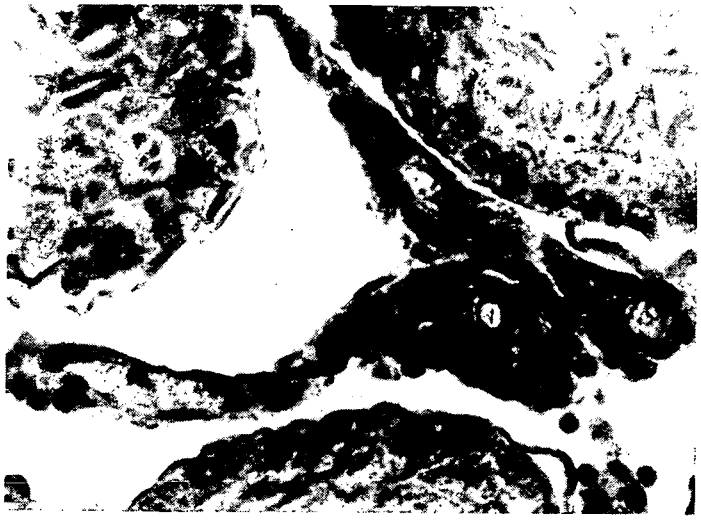

Figure 2. Testes 30 days after irradiation. Destroyed seminal vesicles, lined by a single row of Sertoli's cells. Hematoxylin-eosin, magnification 800 x, ob. 70.

especially behind the anterior commissura alba, accumulation of products of cellular breakdown was observed only on the lateral wall of the ventricles, primarily under the ependyma of their dorsal lateral angles (Figure 5). Beneath the ependyma of the third and fourth ventricles and the aquaeductus sylvii, we did not observe any such demonstrative destructive processes, but beneath the ependyma of the lower horns of the lateral ventricles we found only isolated destructive glial cellular elements. In the cortex of the brain, in the subcortical formations and the medulla oblongata, we found individual cell fragments and cells which were hyperchromic with wrinkled nuclei and oligodendroglia. In the cerebellum, the cells of the grain and many Purkinje* cells were found, while in the olfactory bulbs there were many mitral cells and grain cells.

An additional experiment was conducted to determine the relationship between the magnitude of the radiation dose and the severity of destructive changes in the brain. In this /236

* Editor's Note: These are large branching neurons in the middle layer of the cortex cerebelli.

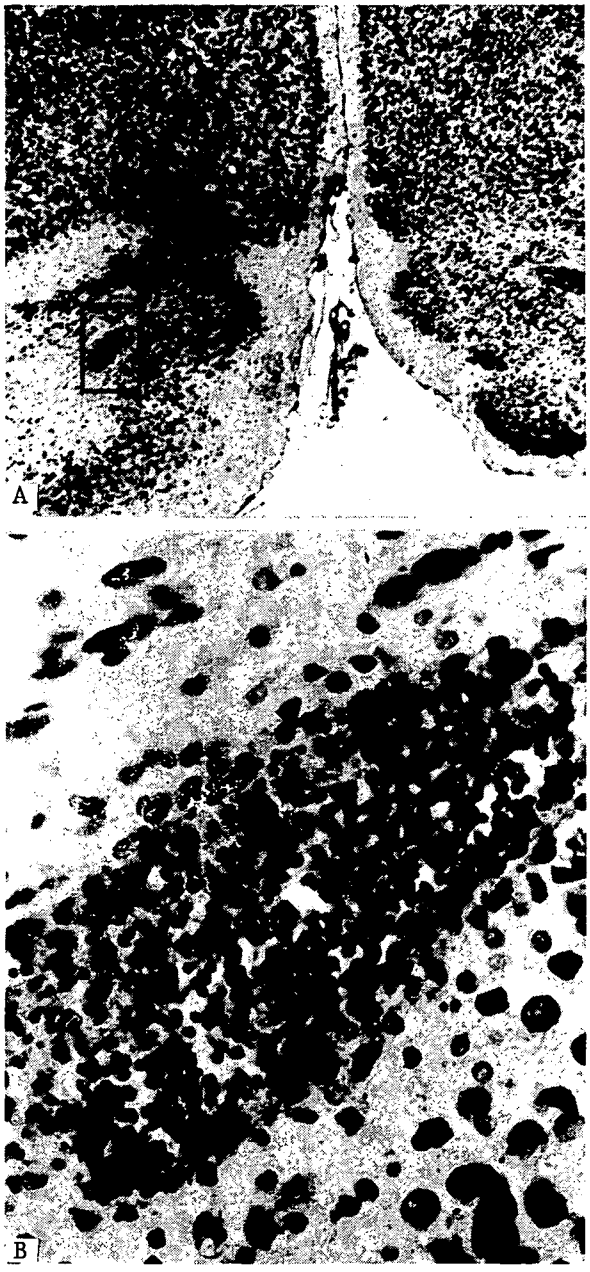

Figure 3. Brain. Frontal section through olfactory and frontal lobes. Sites of maximum accumulation of cell fragments are indicated. Six hours after irradiation. Brache. Magnification 35 x (A); magnification 470 x, ob. 40 (B).

experiment, the rats were subjected to gamma radiation from Co^{60} at doses from 50 to 1000 r. It was found that the minimum dose at which the rats showed death of glial cells was 100 r, and the minimum time following exposure was 2 hours. The higher the dose of radiation, the earlier the breakdown of glial elements began, and the more pronounced and more prolonged the destructive phase became. However, the maximum of these changes always occurred at exactly the same time, 6 to 7 hours following the ionizing effect. The quantity of cell detritus gradually decreased, disappearing almost completely after a few days.

During this same period of time, rats irradiated with neutrons in various parts of the brain including the cerebellum and medulla oblongata showed perivascular edema, vacuolization of the cytoplasm and nuclei of the nerve cells, pulverization and partial dissolution of Nissl substance, hyperchromatoses of individual ganglion cells, wrinkling of their nuclei and the development of cell shadows. At later periods, various portions of the brain mainly showed signs of dystrophy of nerve cells and foci of prolapse.

The picture of cell breakdown in the brain was obtained most clearly following fixation of the material in a Carnot's solution and subsequent Nissl staining, hematoxylin-eosin, methyl green-pyronine. With staining according to Alzheimer and Held, as well as during the development of various forms of glia according to Rio-Hortega and Cajal, the points of maximum accumulation of destroyed cells, in addition to intensively stained and impregnated glial cells, also contained cells and their fragments which accepted the dye weakly. /239

The data obtained indicate that, following a single general gamma neutron irradiation at a dose of 300 rads, rats developed a syndrome of acute radiation sickness of moderate severity with characteristic damage to the hemopoietic organs, intestine, hemorrhagic symptoms, inhibition of spermatogenesis and early destructive changes in the brain.

Irradiation of neutrons with shielding of the stomach or head produces changes in rats which are similar in nature to those obtained when the entire body is irradiated. However, the degree of severity of these changes is much less.

Figure 4. Brain. Sagittal section along anterior horn of lateral ventricle. Products of cell decay in the form of "paths" extend from the olfactory lobe to the frontal lobe. Brache. Magnified with loupe (A). Magnification 240 x, objective 20 (B).

When the stomach is shielded, there is a sharp drop in the damage to the hemopoietic organs. Damage to the follicles of the spleen and foci of myelopoieses of the pulp were much less noticeable (Figure 6), and restoration of their volume was observed earlier. In the marrow, there was no destruction but only impoverishment of the stroma with respect to cellular elements, and the processes of regeneration were more completely evident and terminated at earlier periods. There was a marked decrease in the damage to the intestine. Shortening of the villi of the mucous membrane was much less noticeable and there were almost no signs of atrophy (Figure 7). In the case of unshielded animals,

Figure 5. Brain. Frontal section through interventricular aperture (A). Accumulations of cell fragments are visible (B - enlarged detail). Brache. Magnified 35 x (A); magnified 240 x, objective 20 (B).

six hours following irradiation the mitotic index of the cells in the crypts of the mucous membrane of the intestine fell to zero, while in those rats that were irradiated with protection of the stomach it was only reduced (25.4±3.6%) and returned to the level of the non-irradiated controls in 3 days. At this time, in the unprotected rats, the mitotic activity of the cells in the crypts was reliably higher than the original value, which indicated hyperregeneration (hypercompensation) according to Stround et al. [14]. The mitotic index of the cells in the crypts with shielding of the stomach was increased, in comparison to the original level, only on the 7^{th} and 15^{th} days, but during these times it was reliably lower than

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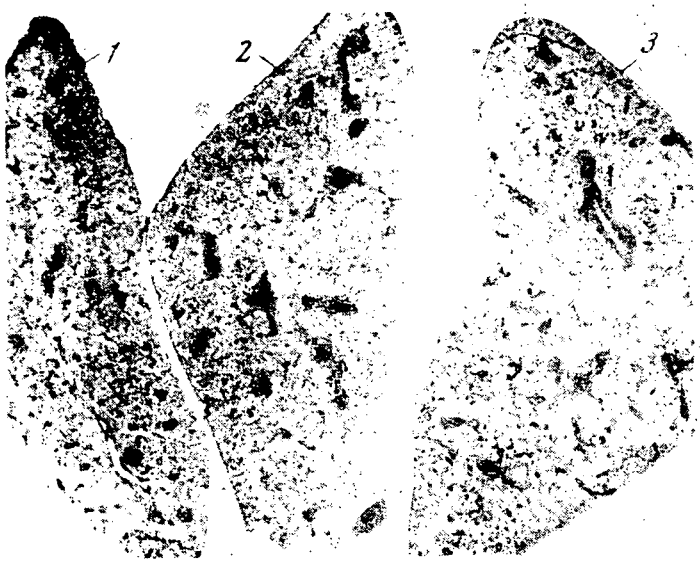

Figure 6. Spleen. Decrease in the size of the follicles and foci of myelopoiesis in unprotected rats (1) with shielding of the head (2) and with protection of the stomach (3). Three days after irradiation. Combined tissue block. Hematoxylin-eosin. Magnified with loupe.

in the unshielded rats. The lymphoid accumulations in the mucous membranes of the intestine of protected rats were restored more rapidly, and after 7 days their number was 3 times greater than in the unshielded rats. All of this indicates that the degree of primary radiation damage to the intestine when it is shielded is decreased considerably, and also leads to a decrease in the functional damage to the intestine. The number of rats in which external signs of diarrhea were observed with shielding of the stomach decreased from 44 to 6%.

There were also differences in the suppression of spermatogenesis. Thirty days afterwards, in those rats that had been subjected to irradiation with protection of the stomach, spermatozoids were contained in almost all of the vesicles. The attenuation of damage to spermatogenesis with shielding of the stomach is also indicated by the fact that after 3.5 and 9 months several of the vesicles in the testes contained all forms /241 of reproductive cells. After 9 months, some of the males had recovered their reproductive function.

Figure 7. Small intestine. Height of villi nearly unchanged. Compare with Figure 1. Three days after exposure with shielding of the stomach. Hematoxylin-eosin. Magnified 90 x, objective 8.

Pathomorphological changes in the brain during the initial periods in terms of nature and degree of severity were the same as in the unprotected animals. At later periods, with partial shielding of the stomach, dystrophically changed cells in various parts of the brain were less frequently encountered.

In the case of irradiation with protection of the head, damage to the organs was more serious than when the shielding was in the vicinity of the stomach, but in these rats also there was a certain decrease in the radiation effect. There was a definite, although less significant, decrease than in the protection of the stomach with respect to the histological changes in the spleen (see Figure 6) and intestine (Figure 8). In these organs, the changes during the majority of intervals occupied an intermediate position between the damage in the unshielded rats and in those animals subjected to irradiation with protection of the stomach. The degree of hemopoiesis inhibition in the marrow and suppression of spermatogenesis in the shielded rats of both series was approximately the same. Direct protection of the head did not have an influence on the changes in the brain, and did not prevent massive breakdown of glial cells under the ependyma of the anterior horns of the

Figure 8. Jejunum. More pronounced shortening of villi than with shielding of the stomach and less pronounced than with irradiation without protection. Compare with Figures 1 and 7. Three days after irradiation with shielding of the head. Hematoxylin-eosin. Magnified 90 x, objective 8.

lateral ventricles. This is explained by the fact that the residual dose behind the shielding was higher than the minimum dose which can produce the changes mentioned above. According to our data, this dose for gamma radiation must be at least 100 r. Inasmuch as the dose behind the shielding with irradiation by neutrons was decreased by 4.6 times, the residual dose was at least 65 rads. Taking the RBE into account for the spectrum of neutrons employed — which according to the data of B. L. Razgovorov in this experiment amounts to at least 2 — the absorbed dose behind the shielding was equivalent to the damaging action of gamma rays and amounted to at least 120 r. However, even under these irradiation conditions, the protective effectiveness of direct shielding was manifested 24 hours after irradiation. At this time, beneath the ependyma of the anterior horns of the lateral ventricles there was restoration of mitotic activity of the glial cells (Figure 9), which according to our data was found in the majority of intact sexually mature rats. At the same time, however, and after 3 days or more, with shielding of the head the cerebral cortex and some of the

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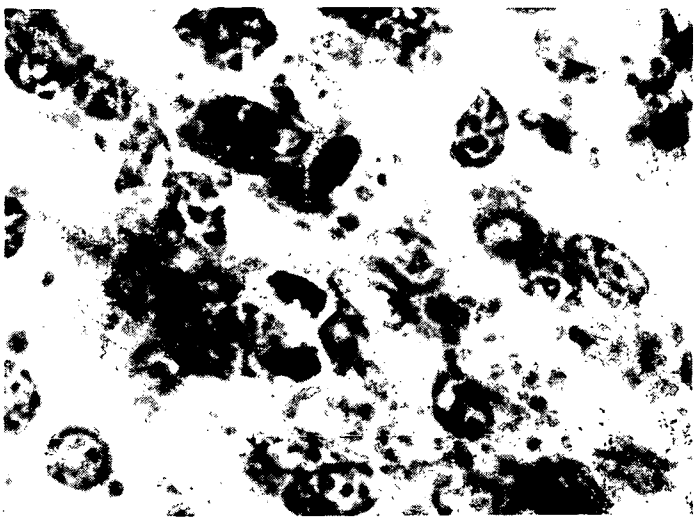

Figure 9. Brain. Mitoses of glial cells in the subependymal layer of the anterior horn of the lateral ventricle. One day following irradiation with shielding of the head. Nissl. Magnified 1250 x, immersion objective 90.

subcortical formations showed a more pronounced satellitosis* reaction of the oliogodendroglia. In addition, there was a decrease in the severity of the dystrophic changes on the part of the nerve cells, which was noticeable to a much greater degree than with shielding of the stomach.

The frequency of hemorrhages and their size in the lungs was less than was noticed in the animals of all three series of irradiations. Apparently irradiation at a dose of 300 rads, with consideration of the dose absorbed by the shielding, was sufficient to cause damage to the convolute and anticonvolute systems of the blood and disruption of the permeability of the vascular walls. /243

Hence, in the case of irradiation by neutrons, shielding of certain parts of the body leads to a decrease in the irradiation effect. The location of the shielding plays an important role as far as the decrease in the damaging effects of ionizing radiation is concerned. The significance of the shielding does not consist merely in the fact that the total dose of radiation is reduced and the damage to the unshielded

* Editor's Note: This is an accumulation of glia cells about the ganglion cells of the brain cortex.

organs is lessened, but it also depends on what part of the body is subjected to the least ionizing radiation. In the case of irradiation by neutrons, there is early death, i.e., mainly "intestinal" death [5, 10]. In the case of unshielded animals, death begins by the 5^{th} day, i.e., during the period of the most severe damage to the intestine when the rats most frequently display diarrhea. When the shielding is located in the vicinity of the stomach, protection of the upper part of the intestine is achieved, which noticeably reduces its damage and thereby prevents the possibility of development of "intestinal" death. Death in these animals occurred at later times, i.e., beginning with the 10^{th} day, and the reason for the death apparently was suppression of hemopoiesis. Attenuation of damage to the spleen by shielding at this radiation dose was insufficient to completely prevent the death of the animals, inasmuch as a second hemopoietic organ, the marrow, was subjected to direct action of neutrons in a considerable portion of its mass.

Judging by the degree of the damaging effect on the intestine, neutrons are quite similar to high-energy protons. In the case of irradiation by protons, death of the animals was observed at early stages as a result of damage to the gastrointestinal tract [8, 11]. It is understandable that shielding of the stomach with irradiation by protons is also an effective means of reducing damage to the intestine and increasing the survival rate of the animals [6]. According to N. F. Barakina [1], the decrease in the radiation effect with shielding of the stomach is explained to a certain extent by the presence in its mucous membrane of factors of a humoral nature, which promote activation of hemopoiesis and the retention of normal absorption.

The positive role of shielding individual organs in reducing their damage has been noticed by a number of investigators.

N. F. Barakina [2] has shown that shielding the spleen with x-irradiation of mice at a dose of 700 rads prevents massive death of hemopoietic cells, caused by the direct action of radiation. With shielding of half the spleen, destructive changes were observed only in the irradiated part of the organ. By shielding /244 the spleen, Jacobson [13] achieved a marked increase in the resistance of mice to x-radiation.

The results obtained and the data in the literature [3, 4, 7] show that shielding of an organ does not insure its absolute protection, due to the remote action of ionizing radiation. The shielded organs will function in the irradiated organisms, and radiation sickness will have a marked influence on their structure and function, especially at later stages, forming pathological processes in the protected organs. A considerable role in the indirect action of ionizing radiation is played by the humoral factor [9]. For this reason, differences in the degree of damage between organs that are shielded against the action of neutrons and those which are not protected gradually become less distinct. Nine months following protection of the stomach, residual changes are found both in the unprotected organs (testes) and in the shielded ones (spleen).

Conclusions

1. A single total gamma neutron irradiation at a dose of 300 rads (neutron contribution to the dose — 90%) causes radiation sickness of moderate severity in rats, characterized by pronounced damage to the intestine in comparison to radiation sickness produced by other forms of radiation.

2. Shielding of limited portions of the body is accompanied by a decrease in the damaging effect of the neutrons. The

location of the shielding is of considerable importance for the attenuation of the radiation effect. Shielding of the stomach has been found to be a more effective method of protection against radiation damage than shielding of the head.

3. With shielding of the stomach against the direct action of radiation, the spleen, upper part of the intestine, liver, part of the marrow (in the vertebrae and ribs) and other organs which are of considerable importance in the formation of the radiation syndrome are protected against the direct action of radiation. A sharp reduction in the damage to the shielded organs was accompanied by less pronounced changes in the marrow and testes, subjected to the direct action of radiation, and insured an increase in the survival rate of the animals during observations for 100 days.

4. With shielding of the head, a decrease in mortality was observed only for 30 days. There was a marked decrease in the damage to the brain. Reduction of damage to the internal organs in subsequent periods was insignificant and was of no importance for the further course of radiation disease.

5. Reduction of the damage to the organs which were not protected against the direct action of radiation (marrow of the femur, testes) was approximately the same, regardless of the location of the shielding.

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Certain Methods of Prophylaxis of Radiation Sickness

L. A. Tyutin

ABSTRACT. Administration of 0.6 g of cystamine hydrochloride to a patient who had undergone radiation treatment prior to each exposure reduces by two to three times the frequency of development of various symptoms of radiation sickness. The prescription of a complex of B vitamins and vitamin C during radiation therapy has a favorable effect on the total condition of the patients, reduces some symptoms of intoxication, but does not have a noticeable influence on the white blood picture.

When using massive doses of ionizing radiation, it is necessary to find means of reducing the effect of radiation on the organism and increasing its protective-accommodative reactions.

From this standpoint, at the present time it is most reasonable to use so-called medical or chemical means of protection, in which the increase in radioresistance of the organism is achieved by administering certain chemical protectors prior to exposure. In a number of studies conducted over the last 10 years, the effectiveness of using certain radioprotective preparations for

prophylaxis of radiation sickness that develops as a consequence of complication of x-ray radiotherapy has been demonstrated [1, 3-8, 10, 11].

Another way of increasing the radioresistance of an organism during the period of radiation treatment is the organization of a rational diet, particularly one which has additional emphasis on vitamin complexes for the patient. A particularly important place is occupied by the B vitamins, the majority of which form a component part of enzymatic systems that play an important role in metabolic processes. As a matter of fact, in a number of papers [2, 3, 8, 9, 13] a decrease in general intoxication was observed as well as a significant improvement in the antitoxic function of the liver in oncological patients who received B vitamins and Vitamin C during the period of radiation treatment.

It should be pointed out, however, that these studies do not exclude all the possibilities of using radioprotective preparations and vitamin complexes in clinical radiology, and they have not been used extensively enough in medical institutions. Therefore, further studies in this direction are justifiable.

At the present time, we are trying to study the effectiveness of using radioprotective preparations of cystamine-hydrochloride in the course of radiation therapy, as well as a complex of B vitamins together with Vitamin C.

This report is based on clinical-hematological observations on 120 patients subjected to radiation treatment under clinical conditions. All of those who were investigated were divided into 3 groups. The first group was the control and consisted of 60 individuals who were given an ordinary hospital diet and

received no radioprotective preparations. Thirty individuals from this group received combined radiation treatment for curing cancer of the uterus, 20 were subjected to post-operative x-ray therapy following extensive extirpation of the uterus with hypophyses and 10 individuals underwent irradiation of the head for treatment of tumors of the pituitary. The second group consisted of 40 individuals who received cystamine in doses of 0.6 g 30 minutes before each exposure; 20 received combined radiation treatment for cancer of the uterus, 10 patients underwent a course of post-operative x-ray therapy following mastectomy to treat cancer, and 10 individuals were irradiated in the area of the head following removal of brain tumors and for treatment of pituitary tumors. During treatment, the patients received 14 to 34 g of cystamine. Finally, in the third group, there were 20 individuals who were suffering from cancer of the uterus (the dose and method of irradiation were the same as for similarly ill patients in the first and second groups). The patients in this group received a combination of B vitamins twice a day in the following doses: B_1 and B_2, 60 mg each; B_6 — 4 mg, PP — 20 mg, folic acid — 2 mg, pantothenic acid — 20 mg, B_{12} — 20 gamma, and Vitamin C — 400 mg.

The radiation treatment was performed on the RUM-11 and RUM-3 x-ray therapeutic apparatus and the GUT-Co-400 gamma-therapeutic apparatus under the following conditions: 180 - 188 kV, 10 - 14 mA, filter 0.5 - 1 mm Cu + 1 mm Al, layer for half-reduction 1.0 - 1.7 mm Cu, skin-focus distance 40 - 50 cm. Gamma therapy was conducted with a skin-focus distance of 35 cm. The basic data on radiation treatment by groups are given in Table 1. As we can see from this table, in the first and second groups the doses and conditions for irradiation show no significant differences, while they were slightly higher for the third group than in the second and control groups.

TABLE 1. BASIC DATA ON RADIATION THERAPY

Diagnosis	Area of irradiation	Form of radiation treatment			Irradiated area, cm^2	Surface dose, r	Dose, absorbed by pathological focus, rads	
		x-ray therapy	Gamma therapy	Combined radiation treatment			From external radiation	From intracavitary irradiation
Cancer of the uterus	Stomach and pelvis	—	—	30	600	10,616 - 13,200	1820-2100	A — 6780-9100
Condition following extirpation of uterus with hypophyses to treat cancer	Pelvis	20	—	—	320	9,372 - 14,616	1730-2800	C — 1800-4650
Tumor of the brain and pituitary	Head	10	—	—	48-240	6000 - 11,050	3000-6000	
Cancer of the uterus	Stomach and pelvis	—	—	20	600	10,870 - 14,900	1890-2700	A — 6730-11,670
Condition following mastectomy to treat cancer	Chest	10	—	—	316-526	13,600 - 16,600		C — 1850-5000
Tumor of the brain and pituitary	Head	—	10	—	48-240	5500 - 11,000	3000-6000	
Cancer of the uterus	Stomach and pelvis	—	—	20	600	11,050 - 15,096	1900-2620	A — 6900-9600 B — 1700-4800

In the course of radiation therapy, the patients in all three groups developed various symptoms of radiation sickness. In order to determine the radioprotective effect of cystamine in the vitamin complex, we compared the frequency of development of certain symptoms of radiation sickness in the investigated groups. The collected data are shown in Table 2. As we can see from this

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TABLE 2. FREQUENCY OF DEVELOPMENT OF CERTAIN SYMPTOMS OF RADIATION SICKNESS DURING TREATMENT

Symptoms	First group (60 individuals) Control		Second group (40 individuals) Patients received cystamine		Third group (20 individuals) Patients received vitamin complex	
	Number	%	Number	%	Number	%
Nausea	22	37	16	40	2	10
Vomiting	2	3.3	—	—	—	—
Disturbance of appetite	40	67	10	25	4	20
General fatigue	51	85	15	38	11	55
Decrease in body weight	25	41	11	27	4	20
Insomnia	18	30	9	22	5	25
Leucopenia (decrease in the number of leucocytes)						
By 25 - 50% relative to original level	31	52	13	32	10	50
By more than 50%	19	31	7	17	7	35

table, nausea developed in the patients of the first and second groups at approximately the same frequency, which may possibly be related to the specific action of cystamine. All of the other symptoms of radiation sickness developed 2 to 3 times less often in patients who received cystamine than in those patients who did not. The decrease in the amount of leucocytes and lymphocytes when cystamine was given was also observed much more rarely and was not so pronounced as in the patients of the control group. Interruptions in the radiation treatment due to persistent leucopenia were made only in the case of 2 patients who underwent irradiation following mastectomy, and displayed high individual sensitivity to ionizing radiation. In the control group, however, treatment was suspended for 5 patients. The symptom complex of radiation sickness of a mild degree in the patients of the control group developed in 20 cases (33%), while in those patients who received cystamine prior to irradiation it developed in only 6 cases (15%). Hence, in our observations, the use of cystamine in the process of radiation treatment turned out to be very effective in the struggle against radiation sickness. /249

The prescription of a complex of B vitamins for the patients as well as Vitamin C had a favorable effect. As we can see from Table 2, the general condition of the patients who received additional doses of the vitamin complex was disturbed to a much lesser degree than in the control group. Nausea developed more rarely in them, and their appetite was better. Eleven persons out of 20 gained weight during treatment. However, it should be pointed out that the use of vitamins did not have a significant effect on the white blood picture. Leuco- and lymphopenia developed in those patients who received the vitamin complex and in the control group to approximately the same degree.

The literature discusses the influence of radioprotective preparations on tumor tissue: do the protectors protect the cancer cells under the influence of ionizing radiation?

Experimental studies [12] as well as clinical observations [1] have shown that radioprotective preparations that prevent the development of radiation sickness or reduce its severity do not reduce the influence of radiation on tumor cells and do not reduce the effectiveness of radiation treatment.

Table 3 presents data on the direct results of combined radiation treatment (administered under equal conditions) in 70 patients with cancer of the uterus.

As we can see from the table, the use of cystamine and the vitamin complex have no influence on the direct results of radiation treatment.

Conclusions

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1. In the course of radiation treatment, oncological patients frequently developed individual symptoms of radiation reactions, and also developed the symptom complex of radiation sickness to a mild degree.

2. Various symptoms of radiation sickness in patients who received the radioprotective preparation cystamine-hydrochloride during treatment developed 2 - 3 times less often than in patients who were subjected to irradiation without chemical protection.

TABLE 3. DIRECT RESULTS OF COMBINED RADIATION TREATMENT OF PATIENTS WITH CANCER OF THE UTERUS

Stage of the disease	Number of patients			Clinical recovery			Clinical improvement		
	1	2	3	1	2	3	1	2	3
1	5	4	2	5	4	2	—	—	—
2	19	13	13	19	13	13	—	—	—
3	3	3	4	2	2	3	1	1	1
4	3	—	1	1	—	—	3	—	1

Note. 1 — first group (control); 2 — second group (received cystamine); 3 — third group (received vitamin complex)

3. The use of a vitamin complex containing B vitamins and Vitamin C during radiation therapy has a favorable effect on the general state of the patients, reducing the signs of intoxication and not having any noticeable influence on the white blood picture.

4. The data which were obtained support the desirability of using cystamine and the vitamin complex from the B group together with Vitamin C during radiation therapy for prophylaxis of radiation sickness.

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IV

COMBINED EFFECT OF IONIZING RADIATION AND NON-RADIATIONAL FLIGHT FACTORS

Reactivity of Irradiated Animals Shielded by Mercapto-(Cystamine and Tsistafos) and Indolylalkylamines (5-MOT and Serotonin) to Transversely Directed Overloads

B. I. Davydov

ABSTRACT. In experiments on 2610 mice, the authors studied the reaction (death) to overloads (44 units) at various periods of radiation sickness at gamma radiation doses from 500 to 4,000 r. Equations of regression were computed for the tolerance of overloads by irradiated animals with the use of radioprotectors. One irradiation: $\log D = 2.993 - (0.055 - 0.072) \cdot t \log e$; cystamine, (75 mg/kg) + irradiation: $\log D = 2.993 - (0.055 - 0.072) \cdot t \log e$; cystamine (150 mg/kg) + irradiation: $\log D = 2.993 - (0.029 - 0.039) \cdot t \log e$; 5-MOT (75 mg/kg) + irradiation: $\log D = 2.993 - (0.036 - 0.043) \cdot t \log e$; serotonin (50 mg/kg) + irradiation: $\log D = 2.993 - (0.049 - 0.067) \cdot t \log e$, where D is the dose of radiation (Rem); t is the time interval after irradiation during which the tolerance of the overload by irradiated animals will not be less than in the controls.

We showed earlier that irradiated animals react to overloads /251 in two phases: in the first phase (1^{st} - 10^{th} days, depending on the dose) there is an increase in resistance and in the second phase there is a decrease in resistance to transversely directed

overloads [3 - 5]. There is a hyperbolic relationship between the degree of resistance to overloads and the dose of radiation: $Dt = 5 \cdot 10^3$, where D is the dose (in Rem) and t is the time following irradiation (in days).

We can assume that radioprotectors which increase the resistance of animals to radiation will also alter their reactivity to transversely directed overloads either lengthening the time of the first phase, i.e., the period of increased resistance, or reducing the severity of the second phase, i.e., normalizing the reactivity during a climax of radiation sickness.

Well-known radioprotective substances were selected from two different chemical groups — the mercapto- and indolylalkylamines: cystamine, tsistafos, 5-methoxytryptamine (5-MOT) and serotonin. The preparations were administered intra-peritoneally in doses (calculated for the base): cystamine — 150 and 75 mg/kg, tsistafos — 300 mg/kg, 5-MOT — 75 mg/kg and serotonin — 50 mg/kg.

The animals were subjected to gamma irradiation (38 r/minute) in doses of 500 - 4000 r and 2 - 40 days after irradiation they were subjected to an overload using the method of fractioned centrifuging [6].

Tolerance to Overloads by Irradiated Animals Protected by Radioprotectors /252

Cystamine. The results of experiments on the influence of this radioprotector on the resistance of irradiated animals to overloads are shown in Tables 1 - 5. Table 1 gives two values for the dynamics of the death of the animals: with consideration

TABLE 1. RESISTANCE OF IRRADIATED (850 r) AND CYSTAMINE-PROTECTED (C = 150 mg/kg) MICE (MALES) TO OVERLOADS.

Group	Interval, days	Number of animals	$P = P_0 + S \lg^* t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
C + 850 r	3	20	$P = 3,63 \pm 0,42 \lg t$	17,2 ±8,6	0,75	0,22
850 r	3	20	$P = 4,16$	20,0 ±9,0	0,89	0,0
		(19)	$P = 3,99$	15,6 ±8,4	0,69	0,0
Control	—	20	$P = 2,35 \pm 1,90 \lg t$	22,6 ±9,5	1,0	1,0
C + 850 r	6	20	$P = 1,45 \pm 2,80 \lg t$	22,6 ±9,5	0,47	1,00
		(18)	$P = 1,85 \pm 5,20 \lg t$	4,9 ±5,0	0,10	1,85
850 r	6	20	$P = 3,98 \pm 1,30 \lg t$	61,0 ±11,2	1,27	0,47
		(10)	$P = 0,56 \pm 3,14 \lg t$	9,7 ±9,5	0,20	1,12
Control	—	20	$P = 2,15 \pm 2,80 \lg t$	48,0 ±11,5	1,0	1,0
C + 850 r	10	20	$P = 3,86 \pm 0,84 \lg t$	38,2 ±11,2	1,57	0,40
		(17)	$P = 3,20 \pm 1,15 \lg t$	25,8 ±10,0	1,06	0,55
850 r	10	20	Survival on the 10th day, 0%			
Control	—	20	$P = 2,20 \pm 2,10 \lg t$	24,2 ±9,7	1,0	1,0
C + 850 r	14	20	$P = 3,81 \pm 1,18 \lg t$	49,6 ±11,0	2,75	0,43

(Continued)

*lg represents log in the above table.

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TABLE 1. RESISTANCE OF IRRADIATED (850 r) AND CYSTAMINE-PROTECTED (C = 150 mg/kg) MICE (MALES) TO OVERLOADS.**

Group	Interval, days	Number of animals	$P = P_0 + S \lg^* t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
850 r	14	(12)	$P = 2,97+1,68 \lg t$	36,2±11,0	2,00	0,61
Control	—	20	$P = 1,24+2,74 \lg t$	18,0±8,7	1,0	1,0
C + 850 r	20	20	$P = 1,54+3,36 \lg t$	46,0±11,5	2,85	1,53
		(18)	$P = 1,46+3,14 \lg t$	34,4±10,9	1,50	1,43
Control	—	20	$P = 1,80+2,20 \lg t$	16,0±8,4	1,0	1,0
C + 850 r	40	20	$P = 3,62+1,18 \lg t$	42,0±11,2	0,82	0,28
		(15)	$P = 1,47+2,94 \lg t$	27,6±12,0	0,53	0,70
C + 750 r	40	20	$P = 2,95+2,00 \lg t$	48,0±11,2	0,93	0,48
		(18)	$P = 2,26+2,42 \lg t$	37,4±11,2	0,73	0,58
700-750 r	40	60	$P = 3,17+1,18 \lg t$	63,8±5,5	1,24	0,28
		(34)	$P = 1,56+2,94 \lg t$	31,0±8,0	0,62	0,70
500-600 r	40	60	$P = 2,90+1,87 \lg t$	45,0±6,0	0,90	0,45
		(58)	$P = 2,75+2,10 \lg t$	44,0±6,1	0,88	0,50
Control	—	20	$P = 1,22+4,18 \lg t$	51,4±10,0	1,0	1,0

(Concluded)

*lg represents log in the above table.

**Commas represent decimal points.

TABLE 2. RESISTANCE OF IRRADIATED (850 r) AND CYSTAMINE-PROTECTED (C = 75 mg/kg) MICE (MALES) TO OVERLOADS**

Group	Interval, days	Number of animals	$P = P_0 + S \lg^* t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
C + 850 r	2	20	$P = 2,38 \pm 1,88 \lg t$	23,0±9,5	0,47	0,86
850 r	2	20	$P = 3,13 \pm 1,34 \lg t$	29,8±8,2	0,60	0,66
Control	—	20	$P = 2,80 \pm 2,18 \lg t$	49,2±11,2	1,0	1,0
C + 850 r	6	20	$P = 3,29 \pm 1,64 \lg t$	47,2±11,2	0,69	0,43
850 r	6	20	$P = 3,86 \pm 1,09 \lg t$	48,0±11,2	0,91	0,29
		(18)	$P = 3,10 \pm 1,55 \lg t$	36,2±11,3	0,69	0,41
Control	—	20	$P = 1,34 \pm 3,78 \lg t$	52,6±11,2	1,0	1,0
C + 850 r	10	20	$P = 4,02 \pm 1,18 \lg t$	58,0±11,2	1,95	0,53
		(13)	$P = 2,16 \pm 2,73 \lg t$	45,6±16,5	1,53	1,23
850 r	10	20	$P = 4,05 \pm 1,55 \lg t$	72,6±10,4	1,82	0,70
		(11)	$P = 1,39 \pm 3,10 \lg t$	30,4±15,0	0,77	1,40
Control	—	20	$P = 2,24 \pm 2,22 \lg t$	29,6±10,5	1,0	1,0
C + 850 r	15	30	$P = 4,62 \pm 1,13 \lg t$	77,2±7,6	2,33	0,53
		(18)	$P = 3,13 \pm 2,23 \lg t$	64,0±11,0	1,93	1,05

*lg represents log in the above table.

(Continued)

**Commas represent decimal points.

TABLE 2. RESISTANCE OF IRRADIATED (850 r) AND CYSTAMINE-PROTECTED (C = 75 mg/kg) MICE (MALES) TO OVERLOADS**

Group	Interval, days	Number of animals	$P = P_0 + S \lg^* t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
Control	—	20	$P = 2,44 + 2,12 \lg t$	33,0±10,8	1,0	1,0
C + 850 r	20	20	$P = 4,9 + 0,88 \lg t$	78,2±9,4	2,26	0,30
		(10)	$P = 3,87 + 1,26 \lg t$	55,0±16,5	1,58	0,42
Control	—	20	$P = 1,70 + 2,96 \lg t$	36,6±11,0	1,0	1,0
C + 850 r	30	20	$P = 5,21 + 0,84 \lg t$	85,2±8,0	2,70	0,27
		(15)	$P = 4,20 + 1,30 \lg t$	69,0±12,5	2,20	0,41
850 r	30	30	Survival on the 30th day, 0 %			
Control	—	20	$P = 1,39 + 3,13 \lg t$	31,4±10,6	1,0	1,0

(Concluded)

*lg represents log in the above table.

**Commas represent decimal points.

Note for Tables 1 and 2. In parentheses — the number of animals surviving at the time of centrifuging; accordingly in graph 4, the analytical expression for overload tolerance is given.

of mortality from irradiation and without it. In Figure 1, we see the dynamics of the mortality of the animals with 10-minute centrifuging. All the control groups (a total of 390 animals) which were in experiments with a radiation dose of 850 r were combined into 1 common group. Reliable limits (p = 0.05) for the death of the control animals with 10-minute centrifuging amounted to 30.3 - 39.7 minutes with an equation of regression

$$P = 2.13 + 2.49 \cdot \log t.$$

TABLE 3. SUMMARIZED DATA ON OVERLOAD TOLERANCE BY MICE IRRADIATED AT A DOSE OF 850 r.**

Group	Time after exposure, days	No. of animals	$P = P_0 + S \lg^* t$	Death (in %) at t = 10 mins.
Control	—	390	$P = 2{,}13 + 2{,}49 \lg t$	35,0±2,5
Experimental	2—3	50	$P = 3{,}42 + 0{,}83 \lg t$	22,6±5,7
		(49)	$P = 3{,}35 + 0{,}83 \lg t$	20,6±5,5
	5—6	50	$P = 3{,}70 + 1{,}06 \lg t$	40,4±6,7
		(38)	$P = 2{,}50 + 1{,}62 \lg t$	19,0±6,1
	10	40	$P = 5{,}23 + 0{,}75 \lg t$	86,0±5,4
		(11)	$P = 1{,}39 + 3{,}10 \lg t$	20,4±15,0

*lg represents log in the above table.
**Commas represent decimal points.

All of the animals that were irradiated with a dose of 850 r without protection, represented in this series of tests, were also combined into one single group (Table 3).

As in our preceding studies, with a single exposure of the animals, they were more resistant during the first few days to overloads. Thus, with irradiation at a dose of 850 r the period of increased resistance was 3.5 - 5 days (Figure 1), which is close to the value obtained by us earlier.

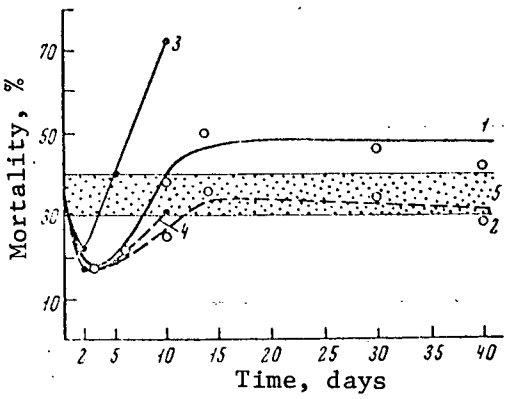

Figure 1. Reaction of irradiated (850 r) mice, protected by cystamine (150 mg/kg) to overloads (44 units, 10 minutes).

1 - shielding + irradiation + overload (with consideration of death from irradiation); 2 - shielding + irradiation + overload (without consideration of death from irradiation); 3 - irradiation + overload (with consideration of death from irradiation); 4 - irradiation + overload (without consideration of death from irradiation); 5 - control (reliable intervals at $p = 0.05$).

TABLE 4. AVERAGE EFFECTIVE TIME (ET_{50} MIN) FOR DEATH OF IRRADIATED (850 r) AND PROTECTED (BY MEANS OF PHARMACEUTICAL PREPARATIONS) ANIMALS DURING CENTRIFUGING.

Time following irradiation, days	Preparation and dose, mg/kg	ET_{50}, minutes
2 - 3	0	81.0 (97)*
5 - 6	0	17.0 (34.6)*
5	Cystamine, 150	81.0
5	Cystamine, 75	11.0
5	Tsistafos, 300	10.0
5	5-MOT, 75	26.0
5	Serotonin, 50	21.0
	Control	14.0

*Numbers in parentheses — without consideration of death from irradiation.

However, if we take into consideration the slope of the line, which in the case of irradiated animals differs sufficiently from the controls, the increase in resistance to overloads during the first 2 - 6 days following irradiation will be more pronounced. Thus, in one of the experiments (see Table 1) we found a zero slope of the line, which indicated the absence of death of animals in the 10^{th}, 15^{th} and 20^{th} minutes of centrifuging. In the combined selection the value of S for 2 - 3 days after irradiation was 0.83, which differs markedly from the control value (S = 2.49). If we calculate the average effective time of death (ET_{50}) during centrifuging, for the controls it will be 14 minutes and for irradiated animals at a dose of 850 r (2 - 3 days after exposure) — 81 minutes (Table 4). Five to six days after irradiation, 76% of the animals survived. Their tolerance to overloads was higher than in the control mice. By the 10^{th} day, survival was 27.5%, but the resistance — if we consider that the basis of its increase to overloads is the selection of the animals in the post-radiation period — was within the limits of the control values.

The animals which received cystamine in doses of 150 and 75 mg/kg prior to exposure, as well as the merely irradiated mice, withstood overloads better during the first days following the exposure. In the protected animals, the period of increased resistance was lengthened to 12 days at a cystamine dose of 150, and at 75 mg/kg it did not differ markedly from that of the irradiated animals (Figures 1 and 2).

In the severity of the second phase (reduced resistance), /256
which begins at a radiation dose of 850 r on the third and fourth days, there are also differences for doses of cystamine of 150 and 75 mg/kg. At a high dose of cystamine, the period of reduced

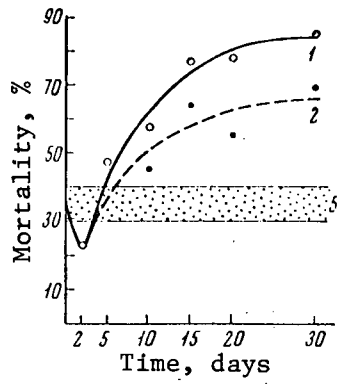

Figure 2. Reaction of irradiated (850 r) mice, shielded by cystamine (75 mg/kg) to overloads (44 units, 10 minutes).

Notation is same as Figure 1.

resistance is less pronounced than with a smaller dose (see Figure 1). The increase in the resistance of the animals to overloads protected by cystamine was observed by us at higher doses as well — 1000 and 2000 r. As we can see from Table 4, two hours after exposure the protected mice withstood the overload better than the control animals. Thus, at a radiation dose of 2000 r death from 10-minute centrifuging was 3.6 $\pm$ 4.3%, while among the controls it was 18.4 + 6.1% ($p < 0.05$).

Tsistafos. The results of tests on the influence of this preparation on the reactivity of irradiated animals to overloads are shown in Table 6. Animals which were shielded against radiation (850 r) by tsistafos were subjected to centrifuging in the 2^{nd}, 5^{th}, 14^{th} and 40^{th} hours following exposure. /258

In contrast to cystamine, this preparation had a different effect on the reaction of the protected mice to overloads. Under the influence of tsistafos, in contrast to cystamine, during the first few days (2 - 10 days) following exposure we did not notice increased resistance, but rather a decrease (Figure 3). Beginning with the 15^{th} day, the resistance of the surviving animals following irradiation reached the control level and remained unchanged for up to 40 days (the last period of observation). The quantitatively determined differences in all periods of

TABLE 5. RESISTANCE OF MICE (FEMALES) TO OVERLOADS TWO DAYS AFTER GAMMA IRRADIATION IN DOSES OF 1000 AND 2000 r AND PROTECTED BY CYSTAMINE (C, 150 mg/kg) AND TSISTAFOS (Ts, 300 mg/kg).**

Group	No. of animals	$P = P_0 + S \lg^* t$	Mortality (in %) at t = 10 mins.	A/A_0	S/S_0
C + 1000 r	17	$P = 3{,}21 \pm 0{,}63 \lg t$	12,2±8,0	0,66	0,37
C + 2000 r	20	$P = 1{,}52 \pm 1{,}68 \lg t$	3,6±4,3	0,19	0,99
Ts + 1000 r	20	$P = -1{,}20 \pm 4{,}50 \lg$	4,5±4,7	0,24	2,65
Ts + 2000 r	20	$P = 1{,}05 \pm 2{,}70 \lg t$	10,6±6,8	0,58	1,60
1000 r	20	$P = 3{,}00 \pm 1{,}05 \lg t$	17,0±8,5	0,92	0,62
2000 r	20	$P = 0$	0	0	0
Control	40	$P = 2{,}41 \pm 1{,}69 \lg t$	18,4±6,1	1,0	1,0

*lg represents log in the above table.

**Commas represent decimal points.

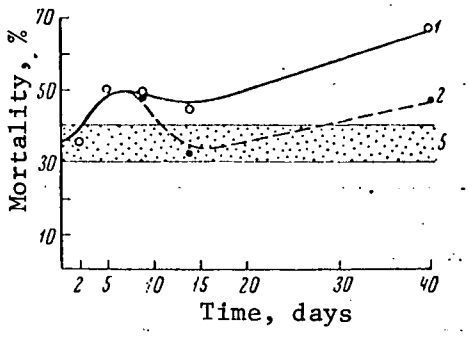

Figure 3. Reaction of irradiated (850 r) mice, protected by tsistafos (300 mg/kg) to overloads.

Notation is same as Figure 1.

TABLE 6. RESISTANCE TO OVERLOADS BY MICE IRRADIATED AND PROTECTED BY TSISTAFOS (Ts, 300 mg/kg) and SEROTONIN (S, 50 mg/kg).**

Group	Interval, days	Number of animals	$P = P_0 + S \lg^{*} t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
Ts + 850 r	2	20	$P = 3,21 + 1,34 \lg t$	32,6±10,7	1,05	0,63
S + 850 r	2	20	$P = 3,38 + 0,79 \lg t$	20,6±9,3	0,67	0,37
Control	—	20	$P = 2,35 + 2,14 \lg t$	30,5±10,5	1,0	1,0
Ts + 850 r	5	20	$P = 3,10 + 1,91 \lg t$	50,2±11,3	1,57	1,18
S + 850 r	5	30	$P = 3,26 + 1,32 \lg t$	33,6±8,5	1,02	0,95
Control	—	30	$P = 3,18 + 1,38 \lg t$	33,0±8,4	1,0	1,0
Ts + 850 r	9	20	$P = 3,00 + 1,38 \lg t$	49,2±11,2	1,40	1,12
		(19)	$P = 3,53 + 1,42 \lg t$	48,0±11,1	1,36	1,15
S + 850 r	9	10	$P = 2,43 + 2,55 \lg t$	49,2±16,5	1,39	2,07
		(9)	$P = 2,35 + 2,60 \lg t$	48,0±16,7	1,36	2,10
Control	—	10	$P = 3,19 + 1,23 \lg t$	35,2±15,7	1,0	1,0
Ts + 850 r	14	10	$P = 2,71 + 2,14 \lg t$	44,2±16,4	2,23	0,75
		(6)	$P = 2,43 + 2,10 \lg t$	32,0±18,0	1,62	0,73
S + 850 r	14	10	$P = 2,66 + 2,14 \lg t$	42,0±16,3	2,12	1,33
		(7)	$P = 0,35 + 4,10 \lg t$	29,0±15,6	1,46	1,44

*lg represents log in the above table (Continued)

**Commas represent decimal points.

TABLE 6. RESISTANCE TO OVERLOADS BY MICE IRRADIATED AND PROTECTED BY TSISTAFOS (Ts, 300 mg/kg) and SEROTONIN (S, 50 mg/kg).**

Group	Interval, days	Number of animals	$P = P_0 + S \lg^{*} t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
Control	—	10	$P = 1{,}30 + 2{,}85 \lg t$**	19,8±13,0	1,0	1,0
Ts + 850 r	40	40	$P = 3{,}73 + 1{,}72 \lg t$	67,2±7,4	1,31	0,41
		(26)	$P = 3{,}40 + 1{,}52 \lg t$	47,0±8,5	0,92	0,36
Ts + 700-750 r	40	60	$P = 3{,}78 + 1{,}12 \lg t$	46,0±6,0	0,90	0,26
		(54)	$P = 3{,}48 + 1{,}28 \lg t$	40,4±5,5	0,79	0,31
S + 850 r	40	20	$P = 3{,}32 + 2{,}28 \lg t$	72,6±11,3	1,40	0,55
		(10)	$P = 2{,}44 + 2{,}46 \lg t$	46,0±16,5	0,90	0,59
Control	—	20	$P = 1{,}22 + 4{,}18 \lg t$	51,4±11,2	1,0	1,0

(Concluded)

*lg represents log in the above table

**Commas represent decimal points.

observation were statistically unreliable for ten minutes of centrifuging. However, it should be pointed out that with $S/S_0 > 1$ and $A/A_0 \geq 1$ in the experimental animals on the 5th ($S/S_0 = 1.18$; $A/A_0 = 1.57$) and 9th days ($S/S_0 = 1.15$; $A/A_0 = 1.36$) the resistance to a more prolonged overload will be statistically reliably lower than in the control mice.

Tsistafos, as we can see, had a different influence on the reactivity of the radiated animals than cystamine had. It is interesting to note that in the study of the toxic effect of these preparations against a background of aftereffects of overload there are also differences [1].

During the first few days following irradiation, cystamine merely lengthens the period of increased resistance of the irradiated animals — in other words, it slightly slows down the development of radiation sickness. However, on the other hand, during the first days following irradiation tsistafos also decreases the resistance of the mice to overloads. We can assume that the phase of reduced resistance in the case of tsistafos begins earlier, i.e., the alternation of the observed state is shifted in phase to the left (compare Figures 1 and 3). It was noted earlier [4] that at the highest doses of radiation the phase of increased resistance is shorter and begins earlier. Hence, it is evident that at the highest doses of radiation we can find a phase of increased resistance even after two days if we assume that tsistafos, like cystamine, merely lengthens it. In Table 5 we have presented the results of experiments on the /259
influence on the reactivity of animals to overloads which have been irradiated at doses of 1000 and 2000 r and protected by tsistafos. At these doses of radiation we see, as in the case of cystamine, the development of a phase of increased resistance of the animals to overloads. However, even in these experiments, /260
there is a more pronounced influence of cystamine on the first phase. Thus, in the case of cystamine the slope angle of the victim line (the value S) is much less than for tsistafos: 0.63, 1.68 and 2.70 and 4.50, respectively. This indicates that with a longer period of rotation the differences between cystamine and tsistafos will be more significant even at radiation doses of 1000 and 2000 r.

5-methoxytryptamine (5-MOT). While the two preceding radioprotectors belong to the group of mercaptoalkylamines, 5-MOT belongs to the indolylalkylamines.

In addition to the practical aspect, the study of this preparation is interesting because in the opinion of many authors [7, 8, 12], amine compounds have a mechanism for radioprotective action which differs from that of thiols.

As we can see from Table 7, the period of increased resistance of irradiated animals protected with 5-MOT at a dose of 75 mg/kg shows little difference from that for cystamine at a dose of 150 mg/kg (Figure 4). It is obvious that the development of the reactivity phases in the irradiated animals protected both by cystamine and 5-MOT basically depends on the phasality of the development of radiation sickness. /262

As in the case of cystamine, the coefficient of the slope of the victim line for resistance of mice to overloads in the case of 5-MOT is less than 1 for approximately 15 to 20 days. Thus, on the 2nd through 15th days, S/S_0 for cystamine and 5-MOT amounts to 0.5 - 0.6. This incidates that with longer centrifuging the difference in the reaction of the irradiated animals protected by these two radioprotectors and the controls will be more significant.

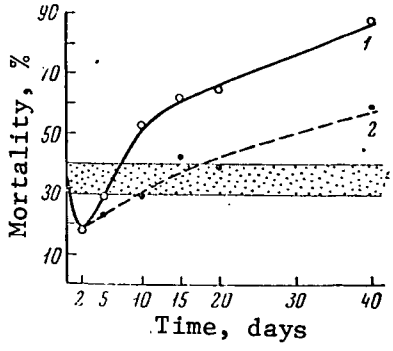

Figure 4. Reaction of irradiated (850 r) mice, protected by 5-MOT (75 mg/kg) to overloads.

Notation is same as Figure 1.

TABLE 7. INFLUENCE OF 5-MOT (75 mg/kg) ON THE RESISTANCE OF IRRADIATED MICE TO OVERLOADS.***

Preparation and irradiation dose	Interval, days	Number of animals	$P = P_0 + S \lg^{**} t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
5-MOT + 850 r	2	30	$P = 2,78 \pm 1,32 \lg t$	18,7±8,9	0,43	0,61
850 r	2	30	$P = 3,25 \pm 1,38 \lg t$	35,4±9,2	0,82	0,64
Control	—	30	$P = 2,66 \pm 2,16 \lg t$	43,0±8,9	1,0	1,0
5-MOT + 850 r*	2	20	$P = -0,55 \pm 4,65 \lg t$	18,4±8,6	0,28	1,57
850 r*	2	20	$P = 1,87 \pm 3,01 \lg t$	45,4±11,2	0,69	1,01
Control*	—	20	$P = 2,44 \pm 2,96 \lg t$	65,6±11,0	1,0	1,0
5-MOT + 500 r	4	20	$P = 2,10 \pm 2,00 \lg t$	18,4±8,7	0,48	0,92
500 r	4	20	$P = 2,12 \pm 2,10 \lg t$	21,8±9,4	0,57	0,97
Control	—	20	$P = 2,54 \pm 2,17 \lg t$	38,4±11,0	1,0	1,0
5-MOT + 500 r	5	40	$P = 3,21 \pm 1,26 \lg t$	29,8±7,2	0,66	0,47
850 r	5	30	$P = 3,90 \pm 0,89 \lg t$	41,2±8,8	0,92	0,33
850 r	5	(28)	$P = 3,20 \pm 1,08 \lg t$	23,6±7,7	0,52	0,40
Control	—	40	$P = 2,18 \pm 2,69 \lg t$	45,0±7,7	1,0	1,0
5-MOT + 850 r	10	30	$P = 4,01 \pm 1,04 \lg t$	52,0±9,0	1,80	0,55

(Continued)

* See the note on Table 2.

** lg represents log in the above table.

*** Commas represent decimal points.

TABLE 7. INFLUENCE OF 5-MOT (75 mg/kg) ON THE RESISTANCE OF IRRADIATED MICE TO OVERLOADS.***

Preparation and irradiation dose	Interval, days	Number of animals	$P = P_0 + S \lg^{**} t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
5-MOT + 850 r	10	(21)	$P = 1,91 + 2,56 \lg t$	30,0±10,5	1,03	1,35
850 r	10	20	$P = 4,05 + 1,55 \lg t$	72,6±10,3	2,48	0,82
850 r	10	(11)	$P = 1,39 + 3,10 \lg t$	30,4±15,0	1,05	1,64
Control	—	30	$P = 2,56 + 1,89 \lg t$	29,0±8,3	1,0	1,0
5-MOT + 850 r	15	30	$P = 3,75 + 1,55 \lg t$	61,6±8,7	1,84	0,73
5-MOT + 850 r	15	(20)	$P = 3,04 + 1,76 \lg t$	42,0±10,2	1,27	0,83
Control	—	20	$P = 2,44 + 2,12 \lg t$	33,0±10,8	1,0	1,0
5-MOT + 850 r	20	40	$P = 4,24 + 1,13 \lg t$	64,4±7,6	1,64	0,50
5-MOT + 850 r	20	(24)	$P = 3,24 + 1,47 \lg t$	38,4±11,0	0,98	0,65
Control	—	40	$P = 2,45 + 2,27 \lg t$	39,0±7,5	1,0	1,0
5-MOT + 850 r	40	30	$P = 4,60 + 1,55 \lg t$	87,4±6,2	2,78	0,50
5-MOT + 850 r	40	(10)	$P = 2,69 + 2,51 \lg t$	58,0±16,4	1,83	0,80
850 r	40	30	Survival on the 30th day, 0 %			
Control	—	20	$P = 1,39 + 3,13 \lg t$	31,4±10,6	1,0	1,0

(Concluded)

*See the note on Table 2.

**lg represents log in the above table.

***Commas represent decimal points.

At higher doses of radiation, 1000 and 2000 r (Table 8), during the first two days there is also an increase in the resistance of the animals to overloads. Statistically reliable differences between protected and unprotected animals were detected only in a dose of 2000 r. Higher resistance to overloads was found in the unprotected animals. The differences between protected and unprotected animals, irradiated with a dose of 4000 r, and the control group of animals were insignificant. At a dose less than absolutely lethal, for example 500 r, 5-MOT had an insignificant effect on the phase of increased resistance of the animals to overloads (see Table 7).

TABLE 8. RESISTANCE OF MICE TO OVERLOADS (MALES) TWO DAYS AFTER IRRADIATION AT A DOSE OF 1000, 2000 AND 4000 r AND PROTECTED BY 5-MOT (75 mg/kg).**

Group	Number of animals	$P = P_0 + S \lg^* t$	Mortality in % at t = 10 minutes	A/A_0	S/S_0
5-MOT + 1000 r	40	$P = -0,21 + 3,89 \lg t$	9,6±4,4	0,39	1,72
1000 r	40	$P = 2,42 + 1,70 \lg t$	19,0±6,1	0,78	0,76
5-MOT + 2000 r	40	$P = 2,40 + 1,50 \lg t$	13,6±5,4	0,56	0,67
2000 r	39	$P = -0,18 + 2,16 \lg t$	0,2±2,0	0,08	0,96
5-MOT + 4000 r	20	$P = 3,23 + 1,25 \lg t$	30,2±10,5	1,24	0,56
4000 r	20	$P = 2,65 + 1,59 \lg t$	22,4±9,4	0,92	0,71
5-MOT	20	$P = 1,04 + 3,06 \lg t$	18,4±8,7	0,76	1,36
Control	60	$P = 2,06 + 2,25 \lg t$	24,4±5,0	1,0	1,0

*lg represents log in the above table.

**Commas represent decimal points.

Serotonin. The results of the experiments testing this radioprotector are shown in Table 6 and in Figure 5. As in the case of the other radioprotectors, serotonin initially produces an increase in the resistance of irradiated animals to overloads with subsequent normalization of reactivity in the surviving animals. The slope of the victim curve (S) is the same as for the other cases, up to five days, and was less than for the control group. Only on the 9^{th} day, when the mortality from irradiation was noticed, did the S/S_0 ratio become greater than 1. /26

Comparative Analysis of the Investigated Radioprotectors

Comparing the various radioprotectors, we notice first of all that at optimally radioprotective doses of cystamine, 5-MOT, and serotonin, the reactions of the protected animals to overloads are similar. A slight exception is tsistafos: it did not cause the development of a phase of increased resistance two to five days after irradiation. The reduction of the dose of cystamine from 150 to 75 mg/kg leads to significant changes in the reaction of the protected animals to overloads: the phase of increased resistance with time remains almost the same as for unprotected animals. The phase of reduced resistance is more pronounced, beginning with the 5^{th} day, and is seen during the entire observation period. /26

The calculation of the average effective time (ET_{50}), i.e., the time of death of 50% of the animals during centrifuging, is shown in Table 4. ET_{50} for cystamine at a dose of 150 mg/kg on the 5^{th} day following irradiation amounted to 81 minutes, i.e., the same as for the non-protected mice, after two to three days. At a dose of 75 mg/kg of cystamine, the ET_{50} on the 5^{th}

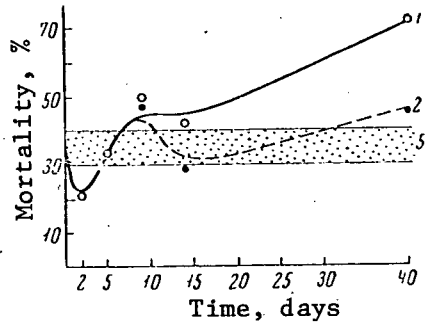

Figure 5. Reaction of irradiated (850 r) mice, protected by serotonin (50 mg/kg) to overloads.

Notation is same as Figure 1.

day amounted to only 11 minutes. Almost the same ET_{50} value was obtained for tsistafos (ten minutes). In the case of 5-MOT and serotonin, this value was almost twice as high: 26 and 21 minutes, respectively. The ET_{50} for control animals was 14 minutes.

Previously [3] we suggested that the increased resistance of animals during the first days following irradiation is due to reduced coagulability of the blood. Fractioned centrifuging made it possible to estimate not only the general resistance of the animals to overload, but also some of its components. In an analysis of the tolerance of the animals to overloads under the influence of radioprotectors, we assume that the coefficient P_0 in the equation of regression describing the victim-logarithmic relationship of the mortality of the animals to the centrifuging time apparently reflects the compensatory capacities of the cardiovascular system in response to overloads. At the same time, the coefficient S represents the accommodative capacities of the organism to ever-increasing hypoxia.

An analysis of the experimental material presented in this paper will show that an increase in resistance to overloads during the first period following irradiation is caused by a decrease in the sensitivity of the organism to increasing hypoxia (a higher value of S in comparison to the control) and to a lesser

degree by the hemodynamic changes caused by reduced coagulability of the blood. However, increased resistance of irradiated rats, particularly to high hypoxia, was observed by many authors [2, 13, 14].

The Irreversible Component of Radiation Damage

As we can see from the experimental data presented above, 30 - 40 days after irradiation the protected animals in some cases showed a decrease in resistance to overloads. We dealt with this earlier as an irreversible component of post-radiation damage, determined by the criterion of tolerance to accelerations. In additional experiments (Table 9), performed on mice exposed to gamma rays and protons (120 MeV) with protection by 5-MOT and /267
tsistafos, we once again demonstrated the existence of an irreversible component which shows up with both gamma and proton irradiation. Figure 6 shows a quite clear relationship between the death of the animals and the dose of gamma radiation (Curve 1). With irradiation using protons, the residual damage determined by the state of reactivity of animals to overloads is much less than for gamma irradiation (Curve 2). From the standpoint of relative biological effectiveness, irradiation with protons in comparison to gamma rays yields a lower-magnitude irreversible component of radiation damage and consequently a lower RBE of the protons, which agrees with the data in the literature [9]. However, in determining the irreversible component on the basis of the repeated irradiation criterion some authors give a value on the order of 20% for the protons and 10% for the gamma rays [10, 11]. This difference may possibly be related to the fact that in the first case we are determining the residual damage suffered by the system in response to the reaction of the animal to the overload (in particular, the cardiovascular system,

TABLE 9. OVERLOAD TOLERANCE BY MICE 30 DAYS AFTER GAMMA IRRADIATION AND IRRADIATION BY PROTONS, PROTECTED BY 5-MOT AND TSISTAFOS.**

Dose, rads	Preparation	No. of animals	Survival on the 30th day, abs	Mortality from overloads of surviving irradiated mice (44 units, 10 mins)		A/A_0
				abs	%	
		Gamma rays, Co^{60}				
0	0	20	20	6	30	1,0
300	0	20	20	9	45	1,50
400	0	20	20	10	50	1,66
500 *	0	100	98	51	52	1,74
600 *	0	100	86	78	90	3,00
		Protons, 120 MeV				
0	0	20	20	7	35	1,0
500	0	20	18	5	28	0,80
600	0	30	10	4	40	1,14
700	0	25	0	—	—	—
500	5-MOT, 70 mg/kg	19	18	5	28	0,80
600	" "	29	16	6	37	1,05
700	" "	30	12	7	58	1,65
500	Tsistafos, 300 mg/kg	20	20	8	40	1,14
600	" "	28	26	13	50	1,42
700	" "	30	24	11	46	1,31

*Double irradiation; first exposure at a dose of 200 r five days before the second — 300 and 400 r.

**Commas represent decimal points.

resistance of the tissues to hypoxia), while in the second we are dealing with all of the systems of the organism, primarily hemopoiesis.

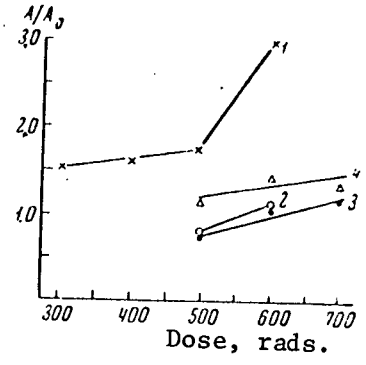

Figure 6. Overload tolerance by mice 30 days after gamma irradiation (1) and irradiation with protons (120 MeV).

2 - single irradiation; 3 - with protection by 5-MOT, 85 mg/kg; 4 - with protection by tsistafos, 300 mg/kg; A - mortality of irradiated animals; A_0 - ditto, for controls. Doses with gamma irradiation at 500 r (200 + 300) and 600 r (200 + 400) were given twice at a 5-day interval.

In the phase of postradiation recovery, the radioprotectors apparently do not play an important role in influencing the reactivity to overloads among the survivors following irradiation of the mice (compare Curves 2, 3 and 4 in Figure 6).

Some Calculations to Determine Tolerable Doses of Radiation (Based on the Criterion of Resistance to Overloads) with Pharmacochemical Protection

Earlier we presented calculations on the tolerated doses of radiation which were determined on the basis of the resistance of animals to overloads [3 - 5]. Now let us use the same method of calculation and attempt to evaluate the tolerable doses using /2
the radioprotectors: cystamine, tsistafos, 5-methoxytryptamine and serotonin. In our calculation, we shall proceed on the basis of the assumption that the preparations affect the duration of the phase of increased resistance of the irradiated animals to overloads. Consequently, the point of intersection of the curve of tolerance in Figures 1 - 5 with the level of tolerance of the control mice will correspond to the value t in the formula $Dt = \text{const}$ (single exposure to overloads) and $D = D_0 e^{-\alpha t}$ (double exposure). With double centrifuging for a single exposure without

pharmacological protection, the following equation of regression was obtained:

$$\log D + 2.993 - 0.065 \cdot t \cdot \log e. \qquad (1)$$

In Figures 1 - 5 we have the data on double centrifuging (twice, 5 minutes each) so that the latter expression is more suitable, which we shall use for evaluating the tolerable radiation doses on the basis of the criterion of resistance to overloads using radioprotectors. /269

Expression (1) is valid for those cases when death from overloads in the controls amounts to 50% on the average [3]. In this series of experiments, with 10-minute centrifuging, the mortality among the controls was 35% (Table 3), and 52% for 15 minutes. For a gamma radiation dose of 850 r or 640 - 720 Rem (RBE = 0.75 - 0.85 [9]) the time of increased resistance (t) with a 15-minute overload will be equal to six days. Following appropriate calculations using Formula (1) the coefficient α at a dose of 640 Rem will be 0.072, while at a dose of 720 Rem it will be 0.055, and the average value for 680 Rem is 0.062. This value is close to the coefficient 0.065 determined previously [5]. Therefore, with some assumptions, we can use expression [1] in this series of experiments.

In the calculation, we used D = 680 (640 - 720) Rem and log D_0 = 2.993. Death of the animals with 15 minutes of centrifuging as a function of the time elapsed following irradiation may be approximated by a linear relationship (Figure 7): $L = a + bT$, where L is the mortality with 15 minutes of centrifuging; T is the time following irradiation (in days); and a and b are coefficients. In Table 10, we have listed the regression equations of overload tolerance in animals protected by cystamine, tsistafos,

TABLE 10. INFLUENCE OF RADIOPROTECTORS ON OVERLOAD TOLERANCE BY IRRADIATED MICE (850 r).

Curve	Protector	Dose, mg/kg	L = a + bT	T*
1	-	0	L = 14 + 6.3·T	6.0
2	Cystamine	75	L = 28 + 4.0·T	6.0
3	Cystamine	150	L = 10 + 3.8·T	11.0
4	Tsistafos	300	L = 19 + 3.8·T	6.3
5	5-MOT	75	L = 19 + 3.8·T	8.7
6	Serotonin	50	L = 12 + 6.2·T	6.5

*Interpolated value of T.

5-methoxytryptamine and serotonin. The values of T were obtained through interpolation. All of the calculations for overload tolerance were performed with consideration of radiation death. The results of the calculations that were performed for the coefficient α in the formula $D = D_0 \cdot e^{-\alpha t}$ are shown in Table 11. As we can see from this table, the most significant increase in the resistance was observed when using cystamine at a dose of 150 mg/kg. After cystamine, come 5-MOT and serotonin. A somewhat unexpected result was obtained in calculations of the tolerable doses with use of tsistafos. However, for a valid evaluation of the use of tsistafos, it must be mentioned that the slope of the line (Figure 7) for this radioprotector is less than for the other preparations. Within the limits of 2 - 14 days, experimental values for tsistafos differ almost insignificantly from the controls. Apparently the levels of tolerable doses may be raised at least to the values for cystamine. /270

TABLE 11. REGRESSION EQUATIONS FOR OVERLOAD TOLERANCE BY IRRADIATED ANIMALS USING RADIOPROTECTORS.

Group	Dose of preparation, mg/kg	t, days (interpolated value)	$\log D = \log D_0 - (\alpha_1-\alpha_2)\cdot t \log e$
Irradiation	0	6.0	$\log D = 2.993 - (0.055-0.72)\cdot t \log e$
Cystamine + irradiation	75	6.0	$\log D = 2.993 - (0.055-0.072)\cdot t \log e$
Cystamine + irradiation	150	11.0	$\log D = 2.993 - (0.029-0.039)\cdot t \log e$
Tsistafos + irradiation	300	6.3	$\log D = 2.993 - (0.051-0.069)\cdot t \log e$
5-MOT + irradiation	75	8.7	$\log D = 2.993 - (0.036-0.043)\cdot t \log e$
Serotonin + irradiation	50	6.5	$\log D = 2.993 - (0.049-0.067)\cdot t \log e$

Note: D is dose of irradiation in Rem; t is the time interval (days) following irradiation during which the tolerance to overloads among the irradiated animals is not less than that of the controls; α_1 and α_2 are calculated, respectively, for doses of 640 and 720 Rem.

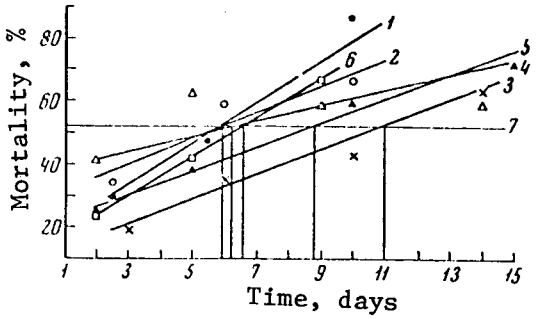

Figure 7. Death of mice with 15 minute overload (44 units) 2 to 15 days after gamma irradiation at a dose of 850 r.

1 - 850 r; 2 - cystamine, 75 mg/kg + 850 r; 3 - cystamine, 150 mg/kg + 850 r; 4 - tsistafos, 300 mg/kg + 850 r; 5 - 5-MOT, 75 mg/kg + 850 r; 6 - serotonin, 50 mg/kg + 850 r; 7 - mortality of control animals.

Previously, by means of extrapolation, we obtained for man doses on the order of 50 - 90 Rem [5]. Obviously we must make a similar attempt to estimate the permissible doses by means of the extremal acceleration tolerance, but under conditions of pharmacochemical protection. /27

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Rate of Recovery of Radioresistance with the Combined Application of Ionizing Radiation and Dynamic Flight Factors on the Organism

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N. N. Dobrov, V. A. Kozlov, V. S. Parshin and P. P. Saksonov

ABSTRACT. In experiments on mice, the authors studied radioresistance recovery rate with combined exposure to radiation and dynamic factors (vibration, acceleration), based on criteria for the change of $LD_{50/30}$ and $AD_{50/4}$ (the dose causing a drop in the weight of the spleen by 50% in four days after the test irradiation). The authors observed a lengthening of the half-recovery period of radioresistance in mice that were subjected to the combined action of radiation and dynamic factors (acceleration, vibration) in comparison to those that were simply irradiated.

Such dynamic flight factors as vibration and overloads may considerably modify the reaction of the organism to irradiation. The trend and magnitude of the changes depend on the nature and force of the stimulus, the time and sequence of action of the factors, the type of biological object, etc. In this connection, there is a very complex interaction between the dynamic and radiation factors, leading in some varieties of experiments to an increase and in others — to a decrease in the resistance of the organism [7, 8, 10, 11, 14, 16].

In their experiments on mice, B. I. Davydov et al. [9] showed that, with irradiation of animals following preliminary /272

acceleration, the $LD_{50/30}$ is increased by approximately 100 r by comparison with a single exposure. Similar results were obtained by Selmer et al. [20] in experiments on rats. A similar effect was observed by T. S. L'vova [17] and Z. I. Apanasenko [4] in the irradiation of animals preliminarily exposed to vibration. However, the course and outcome of the radiation damage in the case of exposure to dynamic factors following irradiation, as a rule, were more serious. This aspect was observed in experiments on lysogenic bacteria [15], drosophila [18] and mammals [5, 11].

The data presented above indicate that, with the combined action of ionizing radiation and dynamic flight factors, the radiobiological effect may change in the direction of an increase or a decrease depending on when these factors act, before or after exposure.

In the majority of investigations studying the biological action of radiation in combination with dynamic flight factors, qualitative methods of evaluating the observed effects are employed.

At the present time, considerable use is being made of the theoretically new method of studying radiobiological reactions which makes it possible to conduct a quantitative estimate of radiobiological effects. It assumes a two-component structure of radiation damage, and determines the quantitative and chronological interrelationships in the irradiated organism between 2 components, i.e. between the restored and irreversible parts of the radiation damage [1, 2, 3, 6, 12, 13, 19].

It seemed to us that the study of these characteristices with the combined action of radiation and dynamic factors would

make it possible to have a quantitative evaluation of the modifying effect of the latter on the reaction radiation course. The principal index of the observed changes was the radio-resistance recovery rate in experimental and control animals.

The experiments were performed on random-bred white female mice. The animals were irradiated on a gamma-cobalt apparatus, the "Khizotron". The intensity of the gamma radiation dose was 12 - 18 r/min.

The combined effect of ionizing radiation and dynamic factors was such that the animals before, after, or before and after irradiation were subjected to the action of acceleration, vibration, or both of these factors. The intervals between each factor will be shown specifically in the description of the experimental results.

The animals were vibrated on a VP-70 type vibrating stand and the VUS-70/200 at a frequency of 70 Hz and a vertical oscillation amplitude of 0.5 mm for 1 hour.

The mice were centrifuged on a centrifuge with a 4.25 m arm. /273
The magnitude of the overload was 30 units. The "launch and descent" lasted 30 seconds each, and the duration of the overload on the "plateau" was 5 minutes.

To evaluate the dynamics of the radiation damage reparation under the combined influence, two criteria were used: (1) the change in $LD_{50/30}$ with two influences and (2) the change in the $AD_{50/4}$ of the dose which produces a decrease in the weight of the spleen by 50% 4 days following the test exposure. The LD_{50} was determined by the victim method.

In the first case, the difference between the total average lethal dose of dual irradiation with different intervals and dosage of initial irradiation (D_1) was used to determine the magnitude of the dose of the second exposure (D_2^t), required to attain the average lethal effect after time t following the first exposure, together with the known first dose. On the basis of the difference between the average lethal dose for a single irradiation and the calculated value found for D_2^t, we could determine the magnitude of the so-called residual radiation damage (D_1^t) for time t (the interval between the first and second exposures).

With consideration of the dynamics of the change in the residual radiation damage with time using the method of least squares, we calculated the period of half-recovery ($T_{1/2}$) of the radioresistance to the repeated irradiation or the combined action of radiation and dynamic factors.

The second method of investigating the dynamics of radiation damage recovery is based on an estimate of the spleen degree of atrophy following irradiation. According to current concepts, the reduction of the weight of the spleen as the principal organ of hemopoiesis in mice bears a definite relationship to the dose of radiation, if the animals for investigation are killed 4 days after the radiation. At sub-lethal radiation doses, there is a good correlation between the severity of the radiation damage and the inhibition of the hemopoietic system.

In our experiments, on the basis of the spleen degree of atrophy, we determined the equivalent doses of radiation for "pure" and combined exposures. Doses which produce 50% atrophy of the spleen ($AD_{50/4}$) were found from the Equation of regression:

$$\log y = a - bx,$$

where y is the weight of the spleen (in %); a and b are constants, and x is the radiation dose ($p \cdot 10^2$).

The duration of the half-recovery period was determined by the least square method on the basis of the change with time in the magnitude of the residual radiation damage. Specific methodical procedures are set forth in the description of the individual experiments.

In studying the dynamics of radioresistance recovery to the combined action of radiation and dynamic flight factors, a series of experiments was performed which encompassed several varieties of temporal relationships in the action of radiation, acceleration, and vibration. /274

In Experiment 1, nine groups of animals were initially irradiated with a dose of 250 r (D_1). To determine $AD_{50/4}$, a second radiation exposure was applied 2, 5 and 15 days after the first. The varieties of the experiments and the results of the experiment are shown in Table 1 and Figure 1. After 4 days following the second exposure, the mice were killed and the values of $AD_{50/4}$ were compared for single and double irradiations. On the basis of the increase in the total dose with double irradiation (single irradiation was also considered to be paired with a zero time interval), we determined the magnitude of the residual radiation damage in the equivalent dose (D_1^t) with time (t) and the dynamics of radioresistance recovery in the controls (only irradiated with the same intervals) and the experimental animals. /275

For groups with repeated irradiation, on the basis of the Equation $AD_{50/4} - 250 = D_2^t$, we found the dose of the secondary (D_2^t) radiation effect which is necessary to cause 50% atrophy of

TABLE 1

INFLUENCE OF THE REPEATED ACTION OF OVERLOADS ON THE RADIORESISTANCE RECOVERY RATE ESTIMATED ON THE BASIS OF THE CHANGE IN $AD_{50/4}$ (Experiment 1)*

Version	Group	Exposure Conditions	$AD_{50/4}$	D_1^t		$D_1^t = D_1 \cdot e^{-bt}$ и $T_{1/2}$
				p	%	
I	1	0	377	--	100	
	2	О —2— О	470	157	62,8	$D_1^t = 100 \cdot e^{-0,199 \cdot t}$
	3	О —5— О	528	99	39,6	
	4	О —15— О	614	13	5,2	$T_{1/2} = 3,48 \pm 0,17$
II	1	0	377	—	100	
	2	П —2— О —1— П —1— О	380	247	98,8	$D_1^t = 100 \cdot e^{-0,116 \cdot t}$
	3	П —2— О —3— П —2— О	511	116	46,4	
	4	П —2— О —13— П —2— О	584	43	17,2	$T_{1/2} = 5,97 \pm 0,18$
III	1	0	377	—	100	
	2	О —1— П —1— О —3— П	424	203	81,3	$D_1^t = 100 \cdot e^{-0,091 \cdot t}$
	3	О —3— П —2— О —3— П	480	147	58,8	
	4	О —3— П —12— О —3— П	559	68	27,2	$T_{1/2} = 7,64 \pm 0,16$

* Commas represent decimal points.

Note: For Tables 1 - 8: П - overload, 0 - irradiation; numbers - time interval between exposures, days; D_1 - dose of initial radiation; D_1^t - magnitude of residual damage in equivalent dose.

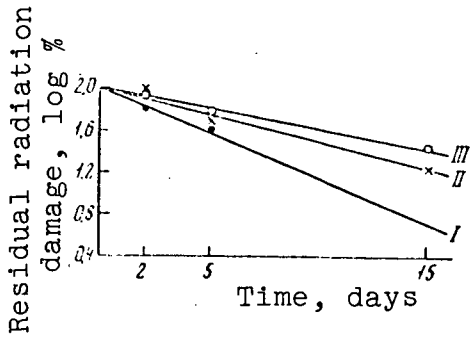

Figure 1. Determination of the half-recovery period ($T_{1/2}$) on the basis of the change in $AD_{50/4}$ with the combined action of radiation and overloads with a time interval of 1 - 3 days. I, II, III, versions of the experiment (Table 1).

the spleen. On the basis of the difference between the dose of 377 r (which causes this effect for t = 0) and D_2^t, we can determine the magnitude of the so-called residual radiation damage D_1^t for time t.

The dynamics of the changes in the residual radiation damage had an exponential character in all varieties of the experiment and were described by the Equation

$$D_1^t = 100 \cdot e^{-bt};$$

the values of b for each variety are shown in Figure 1. It is clear from the latter that, with combined exposure to radiation the period of half recovery $T_{1/2}$ was longer than in version II, only with paired irradiation. This indicates that in this /276
experiment the recovery of the reversible portion of radiation damage is slowed down in animals which have been subjected to repeated exposure to acceleration in selected time combinations with irradiation. The multiplicity of the differences in the duration of the half recovery period in the experimental and control animals was as follows: II: I - 1.72; III : I - 2.19; III : II — 1.28; $p < 0.001$ in all cases. It is important to stress that the maximum lengthening of the half-recovery period was observed in version III, where the first and second overloads followed the irradiation.

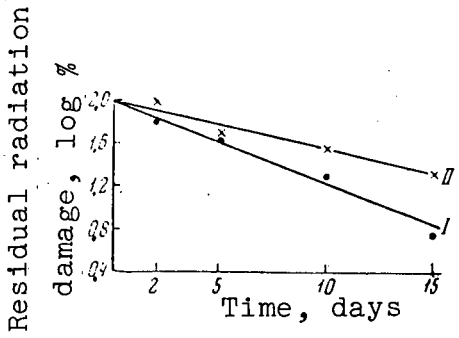

Figure 2. Value of $T_{1/2}$ with the combined influence of ionizing radiation and overloads with an interval of 30 - 50 minutes, determined on the basis of $AD_{50/4}$. I, II, - versions of the experiment (Table 2).

Experiment 2 was performed in a slightly different manner. The animals in version II were subjected to the action of acceleration 50 minutes before and 30 minutes after the first radiation. From Figure 2 and Table 2, we can see that in this experimental arrangement there is a lengthening of the half-recovery period in the animals that are exposed twice to the action of acceleration in conjunction with the first radiation, in comparison with those mice which were merely irradiated. Under these circumstances, the multiplicity of differences of $T_{1/2}$ between the varieties amounted to 1.75 ($p < 0.001$). The coefficient of the difference in this case is close to that which was obtained in version II of experiment 2, where initially the acceleration also ended before irradiation.

The dynamics of the radioresistance recovery in mice subjected to the combined action of radiation and acceleration was evaluated by still another index - the change in the $LD_{50/30}$ with repeated irradiation. For this purpose, four groups of animals were initially irradiated with a dose of 400 r, and then the $LD_{50/30}$ was determined repeatedly after 5 and 15 hours for each experimental and control group of versions I and II. The conditions and results of experiment 3 are shown in Table 3 and Figure 3. /277

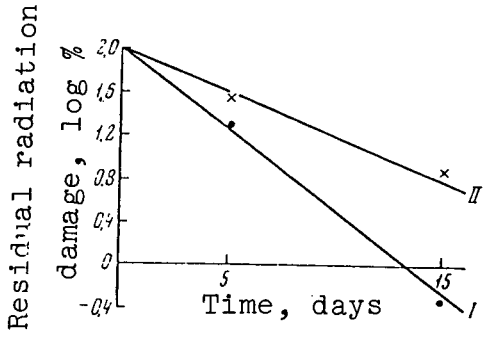

Figure 3. Value of $T_{1/2}$ on the basis of the change in $AD_{50/30}$ with the combined action of radiation and overloads. I, II - versions of the experiment. (Table 3).

We compared the mean lethal dose of single and repeated irradiation in combination with radiation and without it. Judging by the degree of increase in the total dose with paired irradiation (the single irradiation was also considered to be paired, with a zero time interval between irradiations), we determined the residual radiation damage to the organism and the radioresistance recovery dynamics. The residual radiation damage for the investigated periods was calculated the same as in experiment 1.

The period of half-recovery was found to be equal to 2.08 $\pm$ 0.10 for those animals which were merely irradiated (version 1) and 3.65 $\pm$ 0.11 for animals with combined exposure to radiation and acceleration. The multiplicity of the differences in this instance amounted to 1.75 ($p < 0.001$). This indicates that, when evaluating the half-recovery period on the basis of the change in $LD_{50/30}$, there is a marked lengthening of the latter (by 1.75 times) in mice with combined exposure, by comparison with animals which were merely irradiated. The marked negative value for D_1^t in this experiment and similar subsequent experiments indicates that, in the control animals 11 to 13 days following irradiation, a period of increased radioresistance appeared.

Experiment 4 was also performed in order to determine the /27
rate of radiation damage recovery in the period of radioresistance

TABLE 2

INFLUENCE OF REPEATED ACTION OF OVERLOADS WITH SHORT INTERVALS ON THE RADIORESISTANCE RECOVERY RATE, EVALUATED ON THE BASIS OF THE CHANGE IN $AD_{50/4}$. (Experiment 2).*

Exp. version	Group	Exposure conditions	$AD_{50/4}$	D_1^t		$D_1^t = D_1 \cdot e^{-bt}$ и $T_{1/2}$
				p	%	
I	1	0	325		100	$D_1^t = 100 \cdot e^{-0,181 \cdot t}$
	2	$0 \underset{}{\overset{2}{\frown}} 0$	411	164	65,5	
	3	$0 \underset{}{\overset{5}{\frown}} 0$	473	102	40,8	
	4	$0 \underset{}{\overset{10}{\frown}} 0$	526	49	19,6	$T_{1/2} = 3,82 \pm 0,12$
	5	$0 \underset{}{\overset{15}{\frown}} 0$	562	13	5,2	
II	1	0	325		100	$D_1^t = 100 \cdot e^{-0,104 \cdot t}$
	2	ПОН $\underset{}{\overset{2}{\frown}} 0$	326	249	99,5	
	3	ПОН $\underset{}{\overset{5}{\frown}} 0$	457	118	47,2	
	4	ПОН $\underset{}{\overset{10}{\frown}} 0$	484	91	36,4	$T_{1/2} = 6,67 \pm 0,14$
	5	ПОН $\underset{}{\overset{15}{\frown}} 0$	522	53	21,2	

* Commas represent decimal points.

TABLE 3

CHANGE IN RADIORESISTANCE RECOVERY RATE EVALUATED ON THE BASIS OF THE CHANGE IN $LD_{50/30}$ UNDER THE INFLUENCE OF REPEATED ACTION OF OVERLOADS (Experiment 3). *

Exp. version	Group	Exposure conditions	$LD_{50/30}$	D_1^t		$D_1^t = D_1 \cdot e^{-bt}$ и $T_{1/2}$
				p	%	
I	1	О	768		100	$D_1^t = 100 \cdot e^{-0,333 \cdot t}$ и $T_{1/2} = 2,08 \pm 0,10$
	2	О—5—О	1092	76	19,0	
	3	О—15—О	1176	—8	—2,0	
II	1	О	768		100	$D^t = 100 \cdot e^{-0,189 \cdot t}$ $T_{1/2} = 3,65 \pm 0,11$
	2	О—3—П—2—О—3—П	1029	139	37,4	
	3	О—3—П—12—О—3—П	1135	33	8,2	

* Commas represent decimal points.

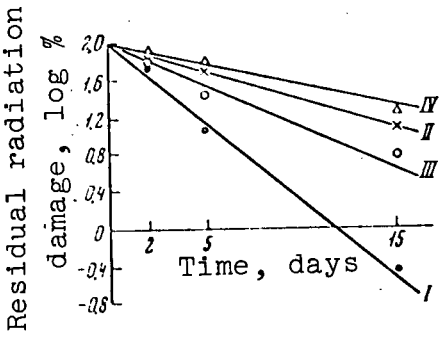

Figure 4. Determination of $T_{1/2}$ on the basis of the change in $LD_{50/30}$ with various combinations of ionizing radiation and acceleration: I - IV - versions of the experiment (Table 4). Symbols are the same as in Figure 1.

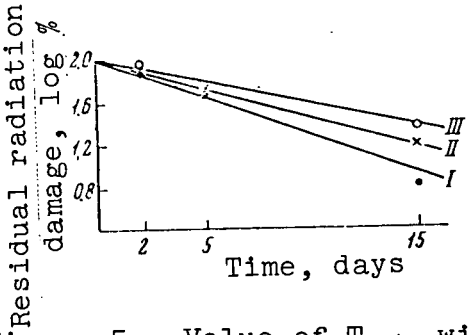

Figure 5. Value of $T_{1/2}$ with combined ionizing radiation and vibration with time interval of 1 - 3 days. I - III - versions of the experiment (Table 5). Symbols same as in Figure 1.

half-recovery on the basis of the change in $LD_{50/30}$ for repeated exposures and for the combined action of radiation and acceleration in the various versions of the experimental arrangement (Figure 4 and Table 4). The method of the calculations is the same as in the preceding experiments.

The animals in the experimental groups in versions II, III and IV were subjected to acceleration for 50 minutes before and 30 minutes after irradiation. It was found that in all experimental groups (versions II - IV) the half-recovery period determined by the change in $LD_{50/30}$ was noticeably longer than in the animals of the control group (version I). The frequency of the differences amounted to the following: between II : I - 2.72; III : I - 1.77; IV : I - 3.71 ($p < 0.001$). Consequently, the maximum lengthening of the half-recovery period was in version IV, where we evaluated the resistance recovery rate not to repeated irradiation, but to repeated combined radiation. In second place as far as the duration half-recovery period is concerned, we find version III, and in third place — version II. /279

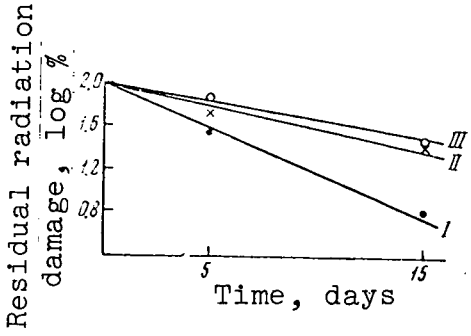

Figure 6. Value of $T_{1/2}$ on the basis of the change in $AD_{50/4}$ with combined radiation and vibration, as well as overloads and vibration with intervals of several minutes. I - III - versions of the experiment (Table 6). Symbols are the same as in Figure 1.

Hence, the results of experiment 4 indicate that the resistance of the organism to repeated irradiation or the combined action of radiation and acceleration is most markedly reduced when the first irradiation is combined with overloads that act before and after the radiation.

A second dynamic flight factor, whose influence on the radioresistance condition with repeated radiation was studied by us, is vibration. Simulation of the combined action /280 of vibration and ionizing radiation was the same as in the experiments with acceleration. This made it possible to compare the experimental results obtained with exposure to both factors separately, and to evaluate later their combined action on the recovery processes in the case of radiation damage.

In experiment 5, the dynamics of the changes in reversible radiation damage with combined exposure to ionizing radiation and vibration, as well as the duration of the half-recovery period, was determined from the change in $AD_{50/4}$ with paired irradiation. The calculation method was standard.

We can see from Table 5 and Figure 5 that, under the influence of vibration as well as under acceleration, there is a slowing down of the post-radiation recovery. The frequency of the differences in the duration of the half-recovery period in the experimental groups of versions II and III, in comparison

TABLE 4

INFLUENCE OF REPEATED EXPOSURE TO OVERLOADS WITH SHORT TIME INTERVALS ON THE RADIORESISTANCE RECOVERY RATE EVALUATED ON THE BASIS OF THE CHANGE IN IN $LD_{50/30}$ (Experiment 4)*

Exp. version	Group	Exposure conditions	$LD_{50/30}$	D_1^t		$D_1^t = D_1 \cdot e^{-bt}$ и $T_{1/2}$
				p	%	
I	1	О	838		100	
	2	О—2—О	1034	204	51,0	
	3	О—5—О	1191	47	11,7	
	4	О—15—О	1250	—12	—3,0	$T_{1/2} = 1,72 \pm 0,13$
II	1	О	838		100	$D_1^t = 100 \cdot e^{-0,148 \cdot t}$
	2	ПОП—2—О	956	282	70,5	$T_{1/2} = 4,69 \pm 0,15$
	3	ПОП—5—О	1046	192	48,0	
	4	ПОП—15—О	1191	47	11,7	
III	1	О	838		100	$D_1^t = 100 \cdot e^{-0,227 \cdot t}$
	2	О—2—ПОП	986	252	63,0	$T_{1/2} = 3,05 \pm 0,13$
	3	О—5—ПОП	1130	108	27,0	
	4	О—15—ПОП	1213	25	6,2	
IV	1	О	838		100	$D_1^t = 100 \cdot e^{-0,109 \cdot t}$
	2	ПОП—2—ПОП	922	316	79,0	$T_{1/2} = 6,37 \pm 0,14$
	3	ПОП—5—ПОП	973	265	66,3	
	4	ПОП—15—ПОП	1165	73	18,2	

* Commas represent decimal points.

TABLE 5

STUDY OF THE INFLUENCE OF VIBRATION ON THE RADIORESISTANCE RECOVERY RATE ESTIMATED ON THE BASIS OF THE CHANGE IN $AD_{50/4}$ (Experiment 5)*

Exp. Version	Group	Exposure Conditions	$AD_{50/4}$	D_1^t p	D_1^t %	$D_1^t = D_1 \cdot e^{-bt}$ и $T_{1/2}$
I	1	O	360		100	
	2	$O\overset{2}{\text{———}}O$	428	182	72,8	$D_1^t = 100 \cdot e^{-0,179 \cdot}$
	3	$C\overset{5}{\text{———}}O$	506	104	41,6	
	4	$O\overset{15}{\text{———}}O$	595	15	6,0	$T_{1/2} = 3,88 \pm 0,14$
II	1	O	360		100	
	2	**$B^1—O^1—B^1—O$	421	189	75,6	$D_1^t = 100 \cdot e^{-0,134 \cdot t}$
	3	$B^1—O^{\backslash}—B^1—O$	487	123	49,2	
	4	$B^1—O^{14}—B^1—O$	575	35	44,0	$T_{1/2} = 5,15 \pm 0,13$
III	1	O	360		100	
	2	$O^1—B^1—O^1—B$	395	215	86,0	$D_1^t = 100 \cdot e^{-0,134 \cdot t}$
	3	$O^3—B^2—O^3—B$	480	130	52,0	
	4	$O^3—B^{12}—O^3—B$	556	54	21,6	$T_{1/2} = 6,55 \pm 0,14$

* Commas represent decimal points.

** Editor's note: Russian symbol B designates vibration (v) in tables 5 - 8.

TABLE 6

INFLUENCE OF REPEATED VIBRATIONS WITH SHORT INTERVALS AS WELL AS VIBRATION AND OVERLOADS ON THE RADIORESISTANCE RECOVERY RATE EVALUATED ON THE BASIS OF THE CHANGE IN $AD_{50/4}$ (Experiment 6)*

Exp. version	Group	Exposure conditions	$AD_{50/4}$	D_1^t p	D_1^t %	$D_1^t = D_1 \cdot^{-bt}$ и $T_{1/2}$
I	1	О	373		100	$D_1^t = 100 \cdot e^{-0,201 \cdot t}$
	2	О—5—О	535	86	34,4	
	3	О—15—О	608	15	6,0	$T_{1/2} = 3,44 \pm 0,16$
II	1	О	373		100	$D_1^t = 100 \cdot e^{-0,100 \cdot t}$
	2	ВОВ—5—О	492	131	52,4	
	3	ВОВ—15—О	559	64	25,6	$T_{1/2} = 6,93 \pm 0,17$
III	1	О	373		100	$D_1^t = 100 \cdot e^{-0,079 \cdot t}$
	2	ПВОВП—5—О	442	181	72,7	
	3	ПВОВП—15—О	551	72	28,8	$T_{1/2} = 8,71 \pm 0,18$

* Commas represent decimal points.

with the control (version I) was as follows: between II : I - 1.33; III : I - 1.69 ($p < 0.001$). /281

This indicates that a more noticeable slowing down of radioresistance recovery occurs when vibration follows the first and second irradiations, and does not precede them. The same relationship was found in analogous versions of experiment 1 when studying the influence of acceleration on the state of radioresistance.

In experiment 6, we studied the influence of vibration on the state of radioresistance with repeated radiation with other time combinations with radiation exposure, and we also studied the radioresistance recovery rate following combined acceleration, vibration, and irradiation. The animals were subjected to repeated irradiation 5 and 15 days after the first exposure.

The time intervals between the dynamic factors and irradiation were as follows:

- In version II the vibration was performed for 45 minutes before the first exposure and for 25 minutes after it;

- In version III, the animals were subjected to acceleration before the first vibration, and after the second with intervals of 3 - 5 minutes.

From the data in Table 6 and Figure 6, we can conclude that with the time relationships selected in this experiment for the action of ionizing radiation and vibration, the period of half-recovery is increased with a combination of two of these factors (version II in comparison to version I). The multiplicity of the differences amounted to the following:

$$\frac{T_{1/2}(II)}{T_{1/2}(I)} = 2{,}01, \quad p < 0{,}001.$$

The period of radioresistance half-recovery was found to be much longer in the animals of version III of the experiment, where the mice were subjected to the action of acceleration and vibration before the first irradiation, and after it with small time intervals between. The multiplicity of the differences in the duration of the half-recovery period between the experimental animals (version III) and the controls (version I) was as follows:

$$\frac{T_{1/2}(III)}{T_{1/2}(I)} = 2{,}53, \quad p < 0{,}001$$

The final experiment, number 7, determining the recovery rate /283
of the reversible component of radiation damage with combined radiation and the magnitude of the half-recovery period was performed using the change in $LD_{50/30}$ with paired irradiation. In version II of experiment 7, the animals were subjected to vibration for 45 and 25 minutes, respectively, before their first irradiation and afterward. The second irradiation was performed 2.5 and 15 days after the first.

In version III of experiment 7, before the first irradiation and after it, the mice were subjected to the action of acceleration and vibration with the same time intervals as in version III of experiment 6.

Version I was the control for paired irradiation. /284

The results of the experiment are shown in Table 7 and Figure 7. We can see from the latter that, even when criteria for changing $LD_{50/30}$ for evaluating the influence of vibration

TABLE 7

RECOVERY RATE OF REVERSIBLE COMPONENT OF RADIATION DAMAGE WITH COMBINED RADIATION AND DYNAMIC FLIGHT FACTORS (OVERLOAD, VIBRATION) (Experiment 7).*

Exp. version	Group	Exposure conditions	$LD_{50/30}$	D_1^t		$D_i^t = D_1 \cdot e^{-bt}$, $T_{1/2}$
				p	%	
I	1	О	834		100	
	2	О $\frac{2}{}$ О	1002	232	58,0	$D_i^t = 100 \cdot e^{-0,403 \cdot t}$
	3	О $\frac{5}{}$ О	1195	39	9,7	
	4	О $\frac{15}{}$ О	1245	−11	−2,7	$T_{1/2} = 1,72 \pm 0,15$
II	1	О	834		100	
	2	ВОВ $\frac{2}{}$ О	939	295	73,7	$D_i^t = 100 \cdot e^{-0,286 \cdot t}$
	3	ВОВ $\frac{5}{}$ О	1157	77	19,2	
	4	ВОВ $\frac{15}{}$ О	1230	4	1,0	$T_{1/2} = 2,42 \pm 0,16$
III	1	О	834		100	
	2	ПВОВП $\frac{2}{}$ О	983	251	62,7	$D_i^t = 100 \cdot e^{-0,233 \cdot t}$
	3	ПВОВП $\frac{5}{}$ О	1125	109	27,2	
	4	ПВОВП $\frac{15}{}$ О	1214	20	5,0	$T_{1/2} = 2,97 \pm 0,16$

* Commas represent decimal points.

TABLE 8

GENERALIZED RESULTS OF EXPERIMENTS IN DETERMINING THE INFLUENCE OF DYNAMIC FACTORS ON THE POSTRADIATION RECOVERY RATE*

Test, $T_{1/2}$	Acceleration					Vibration and vibration in conjunction with acceleration				
	Experiment	Version of experiment	Exposure conditions	$T_{1/2}$	Multiplicity of Differences	Experiment	Version of experiment	Exposure conditions	$T_{1/2}$	Frequency of differences
Change in $AD_{50/4}$	1	I	О—О	3,48		5	I	О—О	3,88	
	1	II	П—О—П—О	5,97	II:I=1,72	5	II	В—О—В—О	5,15	II:I=1,33
	1	III	О—П—О—П	7,66	III:I=2,19	5	III	О—В—О—В	6,55	III:I=1,69
	2	I	О—О	3,8		6	I	О—О	3,44	
	2	II	ПОП—О	6,67	II:I=1,75	6	II	ВОВ—О	6,93	II:I=2,01
						6	III	ПВОВП—О	8,71	III:I=2,53
Change in $LD_{50/30}$	3	I	О—О	2,08		7	I	О—О	1,72	
	3	II	О—П—О—П	3,65	II:I=1,75					
	4	I	О—О	1,72						
	4	II	ПОП—О	4,69	II:I=2,72	7	II	ВОВ—О	2,42	II:I=1,40
	4	II	О—ПОП	3,05	III:I=1,77	7	III	ПВОВП—О	2,97	III:I=1,72
	4	IV	ПОП—ПОП	6,37	IV:I=3,70					

* Commas represent decimal points.

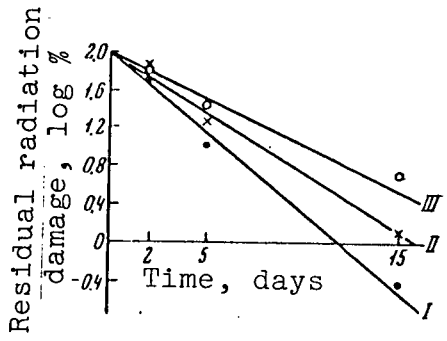

Figure 7. Value of $T_{1/2}$ on the basis of the change in $LD_{50/30}$ with combined ionizing radiation and vibration as well as overloads and vibration with intervals of several minutes. I - III - versions of experiment (Table 7). Symbols are the same as in Figure 1.

and the complex action of vibration and acceleration on the duration of the half-recovery period are employed, the same effect is obtained — the radioresistance recovery rate to the influence of these factors is reduced. This phenomenon is more clearly evident in the case of the complex action of acceleration and vibration prior to irradiation and after it. The frequency of the differences in the duration of the half-recovery period was as follows:

$$\frac{T_{1/2}(II)}{T_{1/2}(I)} = 1.4; \quad p < 0.002;$$

$$\frac{T_{1/2}(III)}{T_{1/2}(I)} = 1.72, \quad p < 0.001.$$

The generalized results of all the experiments determining the radiation damage recovery rate with the combined action on animals of ionizing radiation and dynamic flight factors (acceleration, vibration, and their combination) for various time relationships are shown in Table 8.

It is clear from the latter that, in all varieties of the experiments, we observed a lengthening of the radioresistance half-recovery period in mice subjected to the combined action of radiation and dynamic factors, in comparison with animals which were merely irradiated. This indicates that, under the influence of acceleration and vibration, and also when they are combined, there is a slowing down of the rate of post-radiation recovery. The degree to which the post-radiation recovery rate

is slowed down is determined by the multiplicity of differences between the values of $T_{1/2}$ in the experimental and control animals in the various versions of the experiments. These values are determined by the change in $AD_{50/4}$ and $LD_{50/30}$. As a rule, this recovery rate retardation is expressed to a slightly greater degree in the experiments with acceleration than in those with vibration.

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Influence of Cystamine Combined with Sympathomimetic Amines on Reparative Processes Following the Action of Radiation and Overloads

N. N. Dobrov, V. A. Kozlov, V. S. Parshin, and P. P. Saksonov

ABSTRACT. The experiments were performed on 2410 white mice. Before and after irradiation, the animals were subjected to overloads. As the protector, the authors used cystamine mixed with adrenalin and phenamine. As a criterion for evaluating the protective effect, the reparation rate of the reversible portion of radiation damage was used, determined on the basis of the half-recovery period of radioresistance in the animals. It was found that, with combined radiation, cystamine has a protective effect of approximately the same degree as with a single irradiation.

Under the complex conditions of combined flight factors, /285
there may be changes both in the processes of postradiation recovery and in the nature of the radioprotector influence on the intensity of the reparative processes, etc. This paper is devoted to studying the effectiveness of certain pharmacochemical protective substances with the combined action of ionizing radiation and overloads. The experiments were conducted on 2410 random-bred female mice weighing 20 - 24 grams. As the criteria for evaluating the effectiveness of the radioprotectors, we used the reparation rate of the reversible portion of radiation damage as determined by the radioresistance half-recovery period ($T_{1/2}$) of the animals in paired irradiations.

The period of radioresistance recovery was evaluated by the change /286 in the $AD_{50/4}$ (the dose which causes atrophy of the spleen by 50% on the fourth day after irradiation) and $LD_{50/30}$ (the dose which produces 50% mortality in 30 days).

Irradiation is performed using a gamma-cobalt source at a dose intensity of 12.5 r per minute for 50 minutes prior to irradiation, and 30 minutes afterward the animals were exposed to an overload (15 or 30 units, 5 minutes).

The animals were subjected to acceleration on a centrifuge with a 4.25 m arm. As a radioprotective substance, we used cystamine (100 mg/kg) combined with sympathomimetic amines — phenamine (1.5 mg/kg) and adrenalin (0.15 mg/kg).

It had been established in preliminary experiments that this mixture did not have any negative influence on the tolerance of the animals with respect to overloads, which was observed by using cystamine alone. The medication was administered intraperitoneally 55 minutes prior to exposure, and with combined action — 5 minutes prior to the first centrifuging.

The animals in the experimental groups, following the first irradiation at a dose of 250 r or the combined action of radiation at the same dose and acceleration (30 units, 5 minutes), were subjected after 5 and 15 days to a second exposure at different doses (from 200 to 500 r). We compared the $AD_{50/4}$ for the single and repeated irradiations, which were determined by the equation of regression:

$$\log y = a - bx,$$

where y is the weight of the spleen (in % of control level); x is

the dose of radiation ($p \cdot 10^2$).

Judging by the degree of change in the total dose with paired irradiation (single irradiation was also considered to be paired with a zero time interval between exposures), we determined the residual radiation damage to the organism at periods of 5 and 15 days and the dynamics of radioresistance recovery. The characteristics of the half-recovery period ($T_{1/2}$) in different varieties of the experiments are shown in Table 1. From the data cited, we can conclude that under the influence of overloads the rate of radioresistance recovery as estimated from the periof of half-recovery is slowed down by a factor of more than 2, i.e., there is significant intensification of radiation damage.

When a radioprotector is used prior to irradiation or the combined action of radiation and overloads, the period of half-recovery is shortened significantly. In comparison with the corresponding control, the radioresistance half-recovery period decreases with radiation alone by 1.8 times, while with combined exposure it decreases by 3.2 times. In addition to this experiment, we performed a similar one in which the level of the residual damage to the organism, 2.5 and 15 days following /287
combined action, and the dynamics of radioresistance recovery (judging by the half-recovery period) were evaluated on the basis of the change in $LD_{50/30}$. The radiation dose was equal to 360 r, and the overload was 15 units for 5 minutes.

As in the preceding experiment, in this case we compared /288
the average lethal dose of single and repeated irradiation. These doses were determined with the aid of the victim method. The subsequent calculations were performed the same as in the first experiment. The results of the experiment are shown in Table 2.

TABLE 1

INFLUENCE OF RADIOPROTECTOR ON THE LENGTH OF THE RADIORESISTANCE HALF-RECOVERY PERIOD WITH EXPOSURE TO THE ACTION OF RADIATION AND OVERLOADS ON THE BASIS OF THE $AD_{50/4}$ TEST

Group*	Characteristic of exposure	$T_{1/2}$, days	Result
1	Paired irradiation	4.22±0.22	
2	Protector + paired irradiation	2.32±0.25	Multiplicity of shortening of $T_{1/2}$ relative to the group 1 = 1.82
3	Overload + paired irradiation	9.35±0.17	Multiplicity of lengthening of $T_{1/2}$ relative to group 1 = 2.21
4	Protector + overload + paired irradiation	2.91±0.15	Multiplicity of acceleration of $T_{1/2}$ in comparison with group 3 = 3.21.

*240 animals in each group.

TABLE 2

INFLUENCE OF RADIOPROTECTOR ON THE LENGTH OF THE RADIORESISTANCE HALF-RECOVERY PERIOD WITH EXPOSURE TO RADIATION AND OVERLOADS ON THE BASIS OF THE $LD_{50/30}$ TEST

Group*	Characteristic of exposure	$T_{1/2}$, days	Result
1	Paired irradiation	2.02±0.16	
2	Protector + paired irradiation	1.04±0.13	Multiplicity of reducing $T_{1/2}$ relative to group 1 = 1.94
3	Overload + paired irradiation	4.7±0.18	Multiplicity of increasing $T_{1/2}$ relative to group 1 = 2.32
4	Protector + overload + paired irradiation	2.71±0.14	Multiplicity of reducing $T_{1/2}$ relative to group 3 = 1.74

* 240 animals in each group.

It is apparent from Table 2 that the radioresistance recovery rate of the organism under the combined influence of penetrating radiation and overloads was reduced by a factor of more than 2, i.e., the degree of damage due to radiation was the same as in the preceding series of experiments, as evaluated by the $AD_{50/4}$ test. The use of radioprotector prior to irradiation decreases by approximately a factor of 2 the duration of the radioresistance half-recovery period.

When using substances for pharmochemical protection prior to the combined action of radiation and overloads, the radioresistance half-recovery period was shortened by a factor of 1.7.

The data which we obtained indicate that antiradiation effects of substances for pharmacochemical protection with the combined action of radiation and overloads, as evaluated on the basis of the radioresistance recovery dynamics using $AD_{50/4}$ as well as $LD_{50/30}$ as a test, are approximately the same as with radiation alone.

On the basis of these results, we can conclude that specific radioprotectors have a protective action not only with radiation alone, but also under the conditions of combined effects (radiation plus overload).

In this connection, radioprotectors have a positive influence both with respect to an increase in animal survival rate and in the radioresistance recovery rate of the irradiated organism.

N73-20082

Magnitude of the Irreversible Portion of Radiation Damage with The Combined Action of Radiation and Acceleration

N. N. Dobrov, V. A. Kozlov, V. S. Parshin and N. I. Yezepchuk

ABSTRACT. In experiments on 430 white mice, studies were carried out involving a quantitative determination of the relative value of the irreversible component of radiation damage under the combined action of gamma radiation and acceleration. After a long period of time following preliminary irradiation in sublethal doses up to a total dose of 800 r, or the combined action of radiation in the same doses and acceleration, changes remained in the organism that reduced its radio-resistance and resistance to the combined action of radiation and acceleration. Under conditions of combined radiation and acceleration, the value of the irreversible part of the radiation damage increases by 3.5 - 9%.

The study of the dynamics of the recovery processes has shown that the moment irradiation starts, compensatory-accommodative processes aimed at eliminating radiation damage begin. On the other hand, there are many data indicating the inability or low ability of the organism to repair a certain /289 portion of the radiation damage. This refers primarily to the damage to those tissues which have a low level of physiological regeneration [5, 6]. In addition, the organism is apparently not able to repair those types of radiation damage which have a pathogenetic relationship with the development of leukoses,

with the genetic effects of radiation, and with shortening the lifetime [4].

A number of authors have expressed the opinion that there are at least two basic components of radiation damage. One of them is associated with damage whose reparation is considerably impeded or is impossible [2, 3, 4, 7, 8].

According to the data of Yu. K. Kudritskiy [10], with multiple irradiation the organism develops, on the one hand, powerful accommodative reactions toward continuing radiation, but, on the other hand, it accumulates pathological changes which cannot be completely offset by protective adaptive reactions, so that the death of the animals must necessarily ensue.

V. I. Korogodin and G. G. Polikarpov [9] feel that irreversible radiation damage appears at more remote periods and has to do with the radiation dose. S. Stearner and S. Tyler [11], in experiments with irradiation of large embryos, found that the reversible portion of the damage has a fixed, constant recovery rate, but the irreversible component of the damage is related to the magnitude of the total radiation dose.

The magnitude of the irreversible part of irradiation damage was estimated by H. Blair [7] as 1.5 - 8%, while G. Davidson [8] found it to be equal to 10%, as the average of its possible values in the experiments he analyzed (from 5 to 15%). I. G. Akoyev [1], in special experiments which he conducted, found that the relative value of the irreversible part of the radiation damage in mice amounts to 13 - 15%, and 10.4 - 16.4% in dogs. Allowance for the irreversible portion of irradiation damage is necessary. This view also extends to cases of repeated exposure to radiation in combination with

other factors. In this connection, we considered it advantageous to conduct studies on the quantitative determination of the relative magnitude of the irreversible radiation damage component with the combined action of ionizing radiation and acceleration. The studies were performed in periods following single (control groups) and repeated combined action, when the reparation of the reversible radiation damage had already been completed. For the cumulation of the irreversible radiation damage, we used multiple combined action of overloads with irradiation in non-lethal doses, with intervals of time between exposures that were sufficient for recovery of reversible radiation damage.

A 3-month experiment was performed on 430 white female mice, /290 subjected to total gamma irradiation in the "Khizotron" gamma-cobalt apparatus, with a dose intensity of 14 r per minute. Centrifuging of the animals was performed on a centrifuge with a 4.25 m arm. The magnitude of the overload was 30 units. The acceleration and deceleration lasted 30 seconds each, and the "plateau" lasted 5 minutes. The conditions of the experiment and its results are shown in the table. Doses which lead to 50% mortality of the animals were determined by the method of victim analysis.

In versions I, II and III of the experiment, the mice were irradiated twice with individual doses of 400 r with an interval of 30 days (total dose of 800 r). In versions I and II, the animals were subjected to the action of acceleration 50 minutes prior to exposure and 30 minutes afterward. Thirty-one days following the second combined exposure or irradiation (version III) the animals were subjected to test irradiation to determine the average lethal dose $LD_{50/30}$. In version I the test exposure consisted in a combination of irradiation and the action of acceleration at the same intervals as in the preliminary exposures.

INFLUENCE OF OVERLOADS ON THE MAGNITUDE OF RESIDUAL RADIATION DAMAGE*

Exp. version	Exposure conditions	Total dose of initial and secondary irradiation, r	$LD_{50/30}$ with test-irradiation, r	Magnitude of radiation	Residual damage
				In equivalent dose, r	%
I	$\text{ПОП}\overset{30}{\text{—}}\text{ПОП}\overset{31}{\text{—}}\text{ПОП}$	800	641 ± 10	197 ± 5	24,6 ± 0,6
II	$\text{ПОП}\overset{30}{\text{—}}\text{ПОП}\overset{31}{\text{—}}\text{О}$	800	690 ± 7	148 ± 11	18,5 ± 1,4
III	$\text{О}\overset{30}{\text{—}}\text{О}\overset{31}{\text{—}}\text{О}$	800	716 ± 9	122 ± 9	15,2 ± 1,1
IV	О	—	838 ± 18	—	—

Note: О - irradiation, П - overload, Numbers - time intervals between exposures, days

*Commas represent decimal points.

At the same time as the test exposure of animals in versions I - III, six groups of control animals were subjected to irradiation at various doses (from 500 to 1000 r) to determine the magnitude of the $LD_{50/30}$ with a single irradiation.

On the basis of the difference in the average lethal doses with single irradiation of the animals in versions IV (control) and I - III (experimental), we calculated the radiation damage remaining in the organism (in equivalent dose and in percent) 30 days following the second preliminary irradiation or the combined action of radiation and acceleration.

The results of the experiment make it possible to evaluate /291
the relative magnitude of that radiation damage in the organism whose reparation is extremely difficult or impossible. After a long time has passed following preliminary irradiation in sublethal doses up to a total dose of 800 r, or with the combined action of radiation at the same doses and acceleration, the organism retains changes that reduce its radioresistance and resistance to the combined action of radiation and acceleration, which are equivalent to the damage from acute radiation exposure at doses of 197 r, 148 r and 122 r in versions I, II and III of the experiment. This corresponds to 24.6, 18.5, 15.2% of the cumulative dose of preliminary irradiation.

The results of version III of the experiment (15.2%) are very similar to the data obtained by I. G. Akoyev in studying the magnitude of the irreversible portion of radiation damage in mice which had just been irradiated. As far as the results of versions II and I are concerned, they indicate that, with the combined action of radiation and acceleration, the magnitude of the irreversible portion of the radiation damage in the

organism increases in comparison to that in the case of pure irradiation. The highest percentage of irreparable radiation damage (24.6%) was found in version I of the experiment, where — in order to determine the irreversible component of the radiation damage — the test was complex (irradiation combined with the action of acceleration on the organism). Irreversible radiation changes during the period following recovery of radioresistance were most advantageously produced with the use of strong influences, which placed increased demands on all basic systems of the organism, or long chronic exposures that led to a constant stress on the compensatory-accommodative reactions [3].

In this connection, it is understandable that there is a slight difference in the magnitude of the irreversible component of radiation damage in the first and second versions of the experiment as performed. Obviously, when using a combination of radiation with acceleration as the test effect, it was possible to show the residual radiation damage more clearly than was possible when using irradiation as the sole test exposure.

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N73-20083

Reaction of the Peripheral Blood in Dogs Under the Complex Action of Transverse Overloads and Gamma Irradiation

N. I. Konnova

ABSTRACT. In experiments on 31 dogs, the author studied the influence exerted on the blood system by the complex action of single preliminary transverse overloads and gamma radiation at a dose of 100 or 200 r with intervals between exposures of two hours or 24 hours. With an interval of two hours and a radiation dose of 100 r, the changes in the peripheral blood during the first hours and days were caused mainly by the overload and consisted in neutrophilic leucocytosis, lympho-, eosino- and thrombocytopenia, which were manifested to a much greater degree than in the group with irradiation alone. With an increase of the dose to 200 r, this effect became less pronounced. With an interval between the influences of several days and a dose of 100 and 200 r, the authors found a more rapid and complete restoration of the peripheral blood composition than for irradiation alone.

In a number of studies devoted to the investigation of the /292
combined action of acceleration and penetrating radiation, it has been found that the action of the inertial forces may considerably change the radiobiological effects — in some versions of experiments causing an increase, and in others a decrease of the resistance of the organism to radiation [1 - 9]. In the majority of the works cited, the principal criteria for the biological effect were the survival of the experimental animals and the average survival time. Inasmuch as the blood system plays a critical role in the radiation damage, we felt that it would be interesting

to trace the changes in the peripheral blood of dogs that take place under the influence of overloads and gamma irradiation. The experiments were performed on mongrel male dogs weighing 7 - 14 kg. The animals were exposed to gamma irradiation with Co^{60} on the "Khizotron" apparatus (dose intensity of 3.8 - 7.0 r per minute). The overloads in the chest-back direction were reproduced on a centrifuge with a 4.25 m arm.

Depending on the nature of the effects, all the animals were divided into the following groups:

1. Irradiation with 100 r (control);

2. Overload of 8 units, 3 minutes and irradiation with 100 r 2 hours later;

3. Overload of 8 units, 3 minutes and irradiation with 100 r 1 day later;

4. Irradiation at a dose of 200 r (control);

5. Overload of 8 units, 3 minutes and irradiation with 200 r after 2 hours;

6. Overload of 8 units, 3 minutes and irradiation with 200 r after 1 day;

7. Overload of 8 units, 3 minutes (control).

The experiment involved a total of 31 dogs.

In the peripheral blood, we studied the total amounts of leucocytes and erythrocytes as well as the quantities of reticulocytes and thrombocytes and the hemoglobin content (in grams %). We calculated the leucocyte formula in smears stained according to Romanovskiy. Blood for analyses was drawn from the ear vein one to two times prior to the experiment, and 2.4 hours, 1, 3,

7, 10, 15, 20, 25 and 30 days after the exposure.

As we can see from Figure 1, the dynamics of the total number of leucocytes in the animals of the control group (irradiation at 100 r) was typical for a given dose of gamma radiation. The number of leucocytes in the dogs of this group increased insignificantly 2 hours following exposure. Later there was a gradual decrease in their number to a minimum on the 7th day, and prior to the end of the experiment it remained (with small variations) within the limits of 60 - 85% (Table 1).

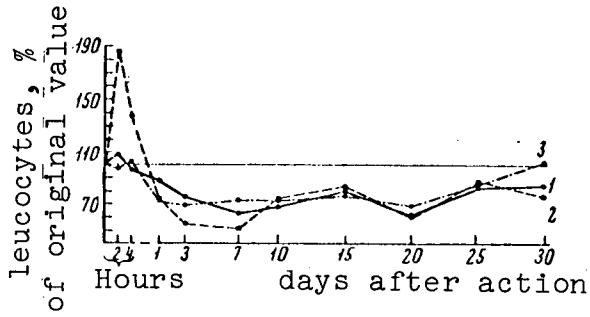

Figure 1. Change in number of leucocytes in the blood of dogs following overloads of 8 units for 3 minutes and irradiation at a dose of 100 r. 1 - irradiation; 2 - irradiation 2 hours following overload; 3 - irradiation 24 hours after overload.

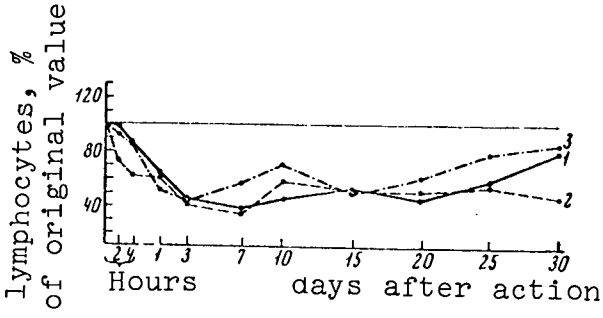

Figure 2. Change in absolute number of lymphocytes in blood of dogs following overloads, 8 units, 3 minutes and irradiation at a dose of 100 r. Symbols are the same as in Figure 1.

In the group in which the animals were subjected to the action of an overload 2 hours prior to radiation exposure, 2 and 4 hours after the radiation we noticed an increase in the total number of leucocytes by 85 and 37%, respectively. Later, the changes in the number of leucocytes were the same as in the control group, with the difference that — in the period from the first to the 7th hours — the leucopenia was more pronounced than in

TABLE 1

DYNAMICS OF CHANGES IN THE PERIPHERAL BLOOD IN SEVEN DOGS FOLLOWING GAMMA IRRADIATION AT A DOSE OF 100 r.*

Irradiation time, days	Thrombocytes, thousands	Leucocytes, thousands	Leucocyte formula, thousands				
			Stabform	Segmented	Eosinophils	Monocytes	Lympho-cytes
Background	227,2	12,1	0,9	6,7	0,86	0,68	2,65
2 hours	215,7	13,0	1,2	7,2	0,69	0,83	2,62
4 hours	308,0	11,5	1,2	6,6	0,35	0,78	2,30
1	300,0	10,6	1,1	4,5	0,71	0,79	1,71
3	244,1	9,1	0,9	4,9	0,97	0,75	1,20
7	221,5	7,7	0,6	4,5	0,74	0,69	1,02
10	130,0	8,2	0,9	4,6	0,77	0,42	1,19
15	136,0	9,7	0,96	6,2	0,42	0,60	1,39
20	220,0	7,4	0,97	4,0	0,46	0,55	1,21
25	248,4	9,9	0,8	5,9	0,60	0,67	1,56
30	336,7	10,2	1,0	5,6	0,62	0,67	2,10

* Commas represent decimal points.

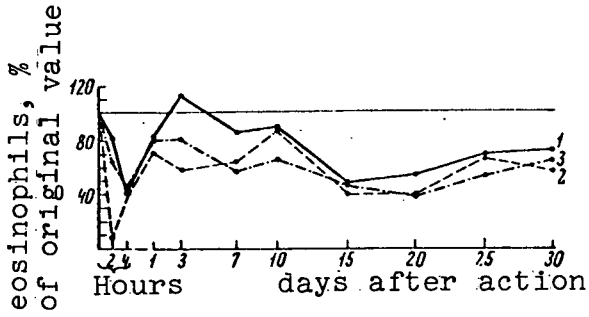

Figure 3. Changes in absolute quantity of eosinophils in the blood of dogs after overloads, 8 units, 3 minutes and irradiation in a dose of 100 r. Symbols are the same as in Figure 1.

the irradiated dogs (Table 2, Figure 1).

With an increase in the interval between exposures up to 24 hours, the nature of the leucocyte reaction changed: there was no leucocytosis during the first hours following exposure. By the 30th day, the level of leucocytes in this group returned to the initial level, which was not observed in the control animals (Table 3, Figure 1).

An analysis of the hemograms showed that the increase in the number of leucocytes that was observed in the dogs of the first and second groups was caused by a rise in the relative and absolute numbers of neutrophils. Neutrophilesis was accompanied by a change in the leucocyte formula to the left, owing to the stabnuclear forms (see Table 1 and 2). The increase in the absolute number of neutrophils 2 and 4 hours following exposure was also observed in the animals of the third group (24-hour interval) in the absence of an increase in the total number of leucocytes in them (see Table 3). At subsequent stages of the examination of the animals of all groups, the change in the absolute number of neutrophils corresponded to the dynamics of the total number of leucocytes. /296

In the animals of the control group (irradiation only), we observed a gradual decrease in the absolute number of lymphocytes, which began 2 hours following irradiation, lasted up to 7 days

TABLE 2

DYNAMICS OF CHANGES IN PERIPHERAL BLOOD IN FIVE DOGS FOLLOWING THE SINGLE ACTION OF TRANSVERSE OVERLOAD (8 UNITS, 3 MINUTES) 2 HOURS BEFORE GAMMA IRRADIATION AT A DOSE OF 100 r.*

Irradiation time, days	Thrombocytes, thousands	Leucocytes, thousands	Leucocyte formula, thousands				
			Stabform	Segmented	Eosinophils	Monocytes	Lympho-cytes
Background	338,0	8,6	0,49	4,8	0,71	0,53	1,83
2 hours	248,0	15,9	2,28	11,2	0,06	0,77	1,32
4 hours	187,0	11,8	1,8	9,1	0,29	0,88	1,14
1	228,0	6,4	0,49	4,0	0,51	0,37	1,11
3	253,0	4,7	0,41	2,7	0,41	0,32	0,74
7	308,0	4,4	0,35	2,7	0,45	0,27	0,62
10	140,0	6,3	0,48	3,6	0,61	0,38	1,07
15	137,0	7,1	0,64	4,8	0,28	0,44	0,94
20	213,0	5,2	0,41	3,2	0,28	0,23	0,94
25	280,0	7,5	0,54	4,7	0,47	0,61	1,0
30	350,0	6,6	0,52	4,4	0,40	0,46	0,84

* Commas represent decimal points.

TABLE 3

DYNAMICS OF CHANGES IN PERIPHERAL BLOOD IN FIVE DOGS FOLLOWING THE SINGLE ACTION OF TRANSVERSE OVERLOADS (8 UNITS, 3 MINUTES) 24 HOURS BEFORE GAMMA IRRADIATION AT A DOSE OF 100 r.*

Irradiation time, days	Thrombocytes, thousands	Leucocytes, thousands	Leucocyte formula, thousands				
			Stabform	Segmented	Eosinophils	Monocytes	Lympho-cytes
Background	271,0	9,3	0,44	4,9	1,61	0,86	1,79
2 hours	182,0	9,1	0,69	5,0	1,03	0,63	1,73
4 hours	200,0	9,5	0,85	5,6	0,72	0,70	1,60
1	305,90	6,8	0,39	3,8	1,28	0,30	0,96
3	331,0	6,5	0,28	3,4	1,28	0,69	0,83
7	271,0	6,8	0,36	3,4	0,92	0,61	1,08
10	144,0	6,8	0,52	3,3	1,05	0,54	1,33
15	182,0	7,2	0,51	4,4	0,73	0,38	0,92
20	280,0	6,5	0,32	3,9	0,62	0,42	1,18
25	327,0	8,0	0,63	2,4	0,85	0,66	1,50
30	222,0	9,6	0,63	5,4	1,03	0,48	1,61

* Commas represent decimal points.

and amounted during this period to 38% of the original value (Figure 2). At subsequent stages, the absolute quantity of lymphocytes remained at a rather low level; only by the 30th day did their number increase and reach 79% (see Table 1).

In the animals which were subjected to the action of overloads 2 hours prior to irradiation, the lymphocyte reaction showed several differences. Thus, during the early stages following exposure (2 and 4 hours) lymphopenia was more pronounced. The level of the number of the lymphocytes at this time was 26 and 24% less than in the controls. In addition, we did not observe any significant differences in the changes of this index in the animals of the experimental and control groups for up to 25 days. By the 30th day, the number of lymphocytes remained at a low level (45% of the original value) and was 34% less than in the control group (see Table 2), where, as we mentioned earlier, the number of lymphocytes increased at this time.

In the group where the interval between exposures was equal to 24 hours, the lymphocyte reaction up until the third day was the same as in the irradiated dogs. Beginning with the 7th day and until the end of the experiment, the absolute number of lymphocytes in the animals that were subjected to complex exposure was somewhat higher than in the controls (see Table 3). Two hours following irradiation, we noticed eosinopenia, the degree of which was different: the smallest decrease in this index was in the animals of the control group (by 18%), and the largest was in the group with the 2 hour interval (by 92%). With a statistical analysis, the differences were bound to be reliable ($p < 0.001$)[1]. In the group where the interval

[1] The statistical processing was done by the Mann-Whitney method.

was equal to 24 hours, the absolute number of eosinophils decreased at this time by 38% relative to the original level. By the 3rd day, the animals of the control group had undergone normalization of this index, and then by the 15th day the number of eosinophils decreased again. In both experimental groups, in the period from the 3rd to the 10th days, the number of eosinophils was lower than in the controls. Beginning with the /297
15th day and until the end of the experiment, the changes in the number of eosinophils in the animals of the experimental groups did not differ significantly from the controls. Normalization of the absolute number of eosinophils had not begun by the 30th day in any of the groups (see Tables 1 - 3, Figure 3).

In the animals of the control group, during a 24-hour period following exposure, we observed an increase in the absolute quantity of monocytes. It was at a maximum 2 hours after exposure, and amounted to 22% of the original level. In the period from the 10th through the 20th days, the number of monocytes decreased by 12 to 39%. Normalization occurred by the 25th day (see Table 1).

In the group with the 2-hour interval, the first hours following exposure saw the absolute quantity of monocytes increase also, but more significantly than in the controls. Thus, their number after 2 hours had increased by 24% and after 4 hours by 66%, which was higher than for these periods in the control animals, by 23 and 51% respectively. However, one day after exposure (and not by the 10th, as in the controls) we observed the start of a decrease in the number of monocytes in the peripheral blood, which was most pronounced on the 25th day of the experiment, when their level amounted to 43% of the initial value. By the 25th day, as in the controls, there was

normalization (see Table 2).

In the animals of the 3rd group (interval = 1 day), only 2 hours after exposure we could already see a decrease in the absolute number of monocytes; their number remained at a low level until the end of the experiment (see Table 3).

In animals of all three groups, we found definite changes in the number of thrombocytes (see Tables 1 - 3). In the dogs of the control group, we observed a typical reaction which took the form of an increase in the blood platelets 4 hours and 1 day following irradiation, at a decrease to 56% of the initial level by the 10th day. The recovery of this index took place by the 20th day.

In the second group (2-hour intervals), we observed a different picture: the number of thrombocytes decreased 2 and 4 hours following exposure by 26.5 and 45%, respectively, from the original values. By the 7th day, the number of thrombocytes had been restored, but by the 10th to 15th days their level again dropped and amounted to 40% of the original value (16% less than in the controls at this time). In addition, all during the experiment the number of blood platelets in the animals of this group was 27 to 34% less than in the controls. Normalization took place only by the 30th day.

In the group where the interval between exposures was equal to 1 day, the differences with respect to the control animals were observed only in the early stages. During the first 2 to 4 hours, we observed a drop in the number of thrombocytes by 34.5 and 26%, while in the control groups thrombocytosis was found. Beginning with the first days and until the end of the experiment, the dynamics of the changes in the number of /298

blood platelets was the same as in the irradiated animals.

In the next three groups of animals, the dose of radiation was increased to 200 r with retention of the intensity and time of exposure of the inertial factor and the intervals between exposures. The data obtained in the animals of this group are represented in Tables 4 - 6 and in Figures 4 - 6.

We can see from Table 4 that irradiation at a dose of 200 r (control group) produced a radiation reaction in the animals which was typical for this dose. The changes corresponded to those described earlier at a dose of 100 r, but these changes naturally were more severe.

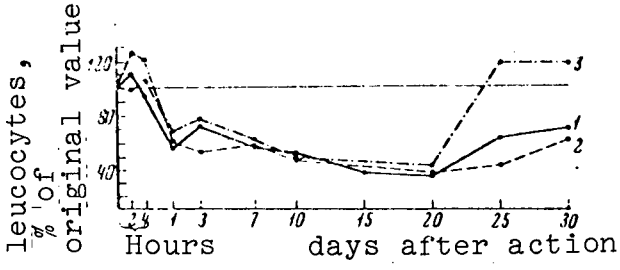

Figure 4. Changes in the number of leucocytes in the blood of dogs following overloads, 8 units, 3 minutes, and irradiation at a dose of 200 r. Symbols are the same as in Figure 1.

In those dogs which were centrifuged 2 hours prior to gamma irradiation at a dose of 200 r, the peripheral blood picture differed slightly from the controls. The total number of leucocytes 2 and 4 hours following exposure increased, and was 16 and 26% higher than in the control dogs. On the 3rd and 25th days, the level of this index was lower than in the controls — by 18 and 20%, respectively. During the remaining periods, we did not observe any important differences in the number of leucocytes in the animals of the experimental and control groups (Figure 4).

An analysis of the morphological composition of the peripheral blood revealed that the changes in the neutrophils corresponded to the changes in the total number of leucocytes.

TABLE 4

CHANGES IN PERIPHERAL BLOOD IN FOUR DOGS FOLLOWING IRRADIATION AT A DOSE OF 200 r.*

Irradiation time, days	Thrombocytes, thousands	Leucocytes, thousands	Leucocyte formula, thousands				
			Stabform	Segmented	Eosinophils	Monocytes	Lympho-cytes
Background	343,8	10,7	0,80	5,6	1,00	0,64	2,65
2 hours	313,7	11,8	1,28	6,6	0,91	0,53	2,20
4 hours	265,8	9,9	0,71	6,3	0,76	0,50	1,54
1	444,4	5,9	0,53	3,6	0,54	0,26	0,72
3	231,2	7,6	0,50	4,3	0,74	0,66	1,15
7	356,2	6,0	0,34	2,9	0,86	0,46	1,06
10	101,0	5,6	0,40	2,3	0,59	0,31	0,95
15	78,0	4,0	0,61	3,3	0,10	0,31	1,06
20	51,0	3,9	0,48	3,0	0,22	0,31	1,16
25	120,6	6,6	0,60	3,8	0,40	0,56	1,40
30	193,4	7,4	0,80	4,3	0,67	0,81	1,56

*Commas represent decimal points.

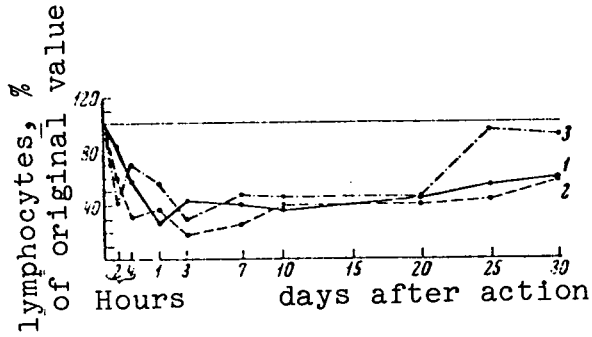

Figure 5. Changes in absolute number of lymphocytes in the blood of dogs following overloads, 8 units, 3 minutes and irradiation at a dose of 200 r. Symbols are the same as in Figure 1.

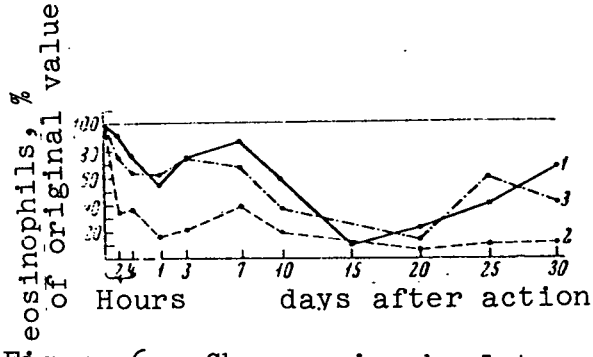

Figure 6. Changes in absolute number of eosinophils in the blood of dogs after overloads, 8 units, 3 minutes, and irradiation in a dose of 200 r. Symbols are the same as in Figure 1.

The absolute quantity of lymphocytes in the animals of the experimental group decreased 2 and 4 hours following exposure by 40 and 69%, respectively, of the original values (23 - 27% less than in the controls). The lymphocyte level in the dogs of the experimental group was less than in the control animals in the period from the 3rd to the 7th days as well. During the remainder of the investigation, the changes in this index in the experimental group were practically the same as in the controls (Figure 5). The absolute quantity of eosinophils in the animals of the experimental group was less during the entire duration of the experiment than in the control dogs (Figure 6). During the first hours following the experiment, the dogs that had been subjected to complex exposure showed an increase in the absolute number of monocytes by 20 - 100%, while their number decreased in the controls at this time (Tables 4, 5). Two and 4 hours following exposure, the dogs of the experimental group showed a decrease in the number of thrombocytes to 53 and 46% of the initial level (115 and 75% in the controls). Following

/300

TABLE 5

DYNAMICS OF CHANGES IN PERIPHERAL BLOOD IN THREE DOGS FOLLOWING THE SINGLE ACTION OF TRANSVERSE OVERLOADS (8 UNITS, 3 MINUTES) 2 HOURS BEFORE GAMMA IRRADIATION AT A DOSE OF 200 r.*

Irradiation time, days	Thrombocytes, thousands	Leucocytes, thousands	Leucocyte formula, thousands				
			Stabform	Segmented	Eosinophils	Monocytes	Lympho-cytes
Background	380,0	10,3	0,77	4,7	0,91	0,65	3,50
2 hours	207,0	12,9	1,37	8,0	0,30	0,78	2,10
4 hours	174,5	12,3	1,36	8,2	0,33	1,30	1,10
1	293,0	6,3	0,44	4,0	0,15	0,30	1,30
3	308,6	5,5	0,40	3,7	0,19	0,43	0,66
7	123,1	5,9	0,78	3,3	0,35	0,31	0,92
10	44,0	4,9	0,41	2,5	0,17	0,21	1,40
20	28,1	3,8	0,23	1,7	0,06	0,28	1,40
25	131,0	4,3	0,20	2,3	0,09	0,20	1,50
30	178,0	6,3	0,48	3,3	0,09	0,33	2,00

* Commas represent decimal points.

normalization of the number of blood platelets, which took place on the third day, the dogs of the experimental group (beginning on the 7th day) showed a renewed decrease in the number of thrombocytes. In the animals of the control group, the decrease in the number of blood platelets began on the 10th day. The regeneration process began in both groups on the 25th day, but by the 30th day none of them had achieved total recovery (see Tables 4, 5).

In the group where the acceleration occurred 1 day prior to irradiation with a dose of 200 r, the reaction of the peripheral blood also differed slightly from that which we found in the control animals. Thus, in the dogs in this group we did not observe an increase in the total number of leucocytes during the first hours following the exposure, but by the 25th day total recovery had occurred (see Figure 4). An analysis of /301
the hemograms showed that — regardless of the fact that the dogs in the experimental group did now show a change in the total number of leucocytes during the first hours following the experiment — the absolute number of neutrophils 2 and 4 hours later increased by 28 and 32%, respectively, relative to the original level, and the number of stabform nuclei increased by 190 - 127% relative to the original levels. On the 25th day, the number of stabform neutrophils* exceeded the original level by 260% (Table 6). The absolute number of lymphocytes in the animals of the experimental group 2 hours following exposure was 42% lower than in the irradiated dogs. During the remaining periods, the number of lymphocytes in the experimental group was slightly higher than in the controls, and by the 25th day, in contrast to the controls, practically complete recovery of the initial level had taken place (see Figure 5). The

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* Editor's Note: This is a neutrophil leucocyte whose nucleus is not divided into segments.

TABLE 6

DYNAMICS OF CHANGES IN PERIPHERAL BLOOD OF THREE DOGS FOLLOWING THE SINGLE ACTION OF TRANSVERSE OVERLOADS (8 UNITS, 3 MINUTES) 24 HOURS PRIOR TO GAMMA IRRADIATION AT A DOSE OF 200 r.*

Irradiation time, days	Thrombocytes, thousands	Leucocytes, thousands	Leucocyte formula, thousands				
			Stabform	Segmented	Eosinophils	Monocytes	Lympho-cytes
Background	271,3	8,9	0,47	5,3	0,00	0,68	1,64
2 hours	305,5	8,7	1,35	6,0	0,45	0,64	0,68
4 hours	301,1	9,3	1,07	6,4	0,38	0,61	1,15
1	167,4	6,0	0,37	4,0	0,37	0,37	0,92
3	208,3	6,8	0,64	3,9	0,45	0,63	0,50
7	68,1	5,4	0,59	3,0	0,41	0,51	0,78
10	41,1	4,3	0,54	2,2	0,21	0,34	0,76
20	125,6	3,8	0,50	2,2	0,08	0,23	0,75
25	190,0	10,4	1,68	6,1	0,35	0,64	1,54
30	334,4	10,4	0,64	7,1	0,24	0,75	1,47

* Commas represent decimal points.

dynamics of the changes in the absolute number of eosinophils and monocytes did not differ significantly from the controls (Figure 6, Tables 4, 6). Two and 4 hours following exposure, the dogs in the experimental group (see Table 6) showed an increase in the number of thrombocytes by 46 - 34% relative to the initial levels. The period of thrombocytopenia was shorter than in the control animals and lasted from the 7^{th} to the 10^{th} days. The process of regeneration had begun on the 25^{th} day and by the 30^{th} day the number of blood platelets exceeded the original level by 25%.

The changes in the red blood in the animals of all the experimental and control groups did not exceed the limits of physiological variation during the entire experiment.

An analysis of the results obtained showed that the changes in the peripheral blood under the conditions of our experiments depended on the dose of radiation and the interval between the action of two agents. Thus, in those groups where the acceleration acted 2 hours prior to gamma irradiation, in the first hours and days the dynamic factor was dominant. As a matter of fact, following exposure of a single overload (8 units for 3 minutes), the dogs showed a reaction in the peripheral blood which took the form of leucocytosis of a neutrophilic nature with a leftwise shift to stabform types, lymphocytosis with subsequent lymphopenia, eosino- and thrombocytopenia (Figure 7). In other words, a picture was seen which was characteristic of a stress reaction. The maximum value of the change was reached 2 hours following the exposure to overload, and normalization took place in 24 hours. Hence, under the influence of the two investigated factors with an interval of 2 hours, irradiation took place on the level of changes that were produced by the overload. This also governed the reaction

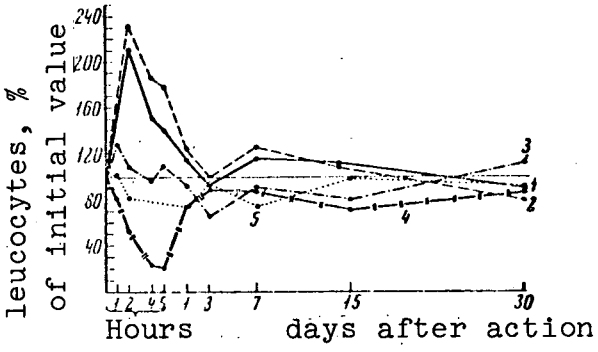

Figure 7. Number of leucocytes (1), neutrophils (2), lymphocytes (3), eosinophils (4) and thrombocytes (5) in the blood of dogs following a single overload, 8 units, 3 minutes.

of the peripheral blood during the first hours and days following exposure. This was most clearly evident in the group where the dose of radiation amounted to 100 r. With an increase in the dose to 200 r, the dominant effect of the dynamic factor on the leucocyte reaction was weakened, and the influence of radiation was stronger. At later stages, the changes in the peripheral blood in the dogs of the experimental groups did not differ significantly from the control with irradiation, and was determined mainly by the radiation factor. It should be pointed out, however, that in those animals that were centrifuged 2 hours prior to irradiation, there was a slight tendency toward a lower content of regular elements in the peripheral blood than in the controls, especially with irradiation at a dose of 200 r. /302

In those groups where acceleration occurred 24 hours prior to irradiation at doses of 100 or 200 r, the reaction of the peripheral blood in the early stages did not differ significantly from the controls. In our opinion, it is interesting to note that in the animals of these groups, at later stages of observation, the absolute number of lymphocytes was higher than in the control animals. By the 25^{th} to 30^{th} days, the composition of the peripheral blood had essentially normalized, which was not seen either in the control groups or in

the groups with the two-hour interval.

Hence, the data we obtained indicate that preliminary exposure to overloads modifies the reaction of the blood system to irradiation in dogs. The modifying action of the overloads on the radiation effects are complex and diverse. The cumulative action of the two investigated factors must be determined on the basis of modern concepts regarding the phasality of the stress reaction. It is obvious that irradiation 2 hours following exposure to acceleration is an additional stress on the organism, constituting a load on all the protective-accommodative and compensatory capacities, and in the final analysis determines the deeper and more long-lasting changes in the peripheral blood. At the same time, however, the use of an /303
overload several days prior to irradiation slightly decreases the irradiation reaction, as indicated by the indices of the peripheral blood. This is apparently explained by the fact that 24 hours following centrifuging the functional and protective capacities have normalized to a certain extent and the irradiation takes place under more favorable conditions.

Hence, the reaction of the organism to the combined action of two factors (overload and irradiation) cannot be determined by the simple summation of their effect. It is also influenced primarily by the fact that both of the factors are combined in intensity and in time.

The data presented do not fully clarify the mechanisms of the changes in the peripheral blood that arise under the complex influence of these factors on the organism. To a certain degree, this can be established by a simultaneous study of the state of the hemopoiesis centers and changes in the metabolism of a number of hormones, enzymes and mediators that

play an important role in the stressor reactions in the adaptative processes of the organism.

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Evaluating the Influence of Vibration on the Radiation Reaction in Dogs with the Aid of Certain Clinical-Hematological Indices

T. S. L'vova

The experiments were performed on 33 mongrel male dogs. The animals were vibrated (70 Hz, 0.4 cm amplitude) for a single period prior to irradiation with an interval between exposures of 2 or 24 hours.

It was shown that the vibration changes the reaction of the peripheral blood to irradiation. The effect of vibration was most clearly evidenced in the group with the two-hour interval between the exposures on the first day of observation. The increase in the radiation dose to 200 r slightly reduced the effect of vibration. The increase of the interval between exposures to 24 hours both at the 100 r and 200 r doses led to the disappearance of the vibration effect.

In experiments with mammals, the modifying effect of vibration on the course and outcome of radiation sickness has been observed. It is evident in the capacity of vibration to both attenuate and intensify radiation damage [8, 9]. However, insufficient attention has been devoted to the changes in the hemopoietic system. In addition, it has been shown that

vibration can produce significant changes of a functional and morphological nature in the hemopoietic organs [1 - 4, 6] which in all probability has some influence on the reaction of this system to radiation. In the present paper, the evaluation of the combined action of several factors was performed with the aid of a number of hematological tests in which the accent was placed on studying the changes in the peripheral blood during the period of the early reaction. Experiments were performed on 33 mongrel male dogs, weighing 8.5 - 9.0 kg. The animals were subjected to vibration for a single period prior to irradiation. The intervals between exposures were 2 and 24 hours. Vibration was carried out on a vibration stand at a frequency of 70 Hz for 1 hour, with an amplitude of 0.4 centimeters. The action of the vibration was combined with gamma radiation at a dose of 100 and 200 r. The influence of the vibration on the radiation reaction was evaluated on the basis of the change in the peripheral blood indices, weight, and also certain clinical data.

The blood was collected from the animals' ear veins at the following periods: on the day before the exposure, and 2, 4 hours and 1 - 30 days after irradiation. The blood indices were studied by a generally accepted method. Simultaneously with the study of the blood, the weight and clinical condition of the animals were observed.

All the animals were divided into the following groups: the first and second groups contained those animals which were subjected to irradiation alone (100, 200 r). The third group consisted of those animals which were subjected to the action of vibration. The fourth and fifth, sixth and seventh groups contained those animals in which combined action of vibration /305

and gamma radiation at doses of 100 and 200 r with intervals of 2 and 24 hours between exposures were studied.

From the data presented above, it is evident that the dynamics of the changes in the peripheral blood in the irradiated dogs were typical of radiation damage (Table 1). However, in those groups where the animals were subjected to vibration with subsequent irradiation, the dynamics of the changes in the peripheral blood showed a number of characteristics which basically occurred in the course of the first days of observation. The most characteristic in this respect was the group with a 2-hour interval between exposures, where the radiation dose was 100 r (Figure 1).

In this group, the animals showed an increase in the number of leucocytes 2 and 4 hours after irradiation by 55 and 35%, respectively, relative to the original level. A comparison of these indices with the controls for radiation at the same observation intervals revealed a statistically reliable difference (after 2 hours — 51%, and after 4 hours — 42%) (Table 2). By the first day, the number of leucocytes in the experimental animals was the same as that in the controls. During subsequent periods, both the experimental and control groups showed a decrease in the number of leucocytes. However, the experimental group showed a tendency toward a less pronounced decrease in the total number of leucocytes.

An analysis of the absolute numbers of individual forms of /30
leucocytes in this group revealed that the increase in the number of cells was caused by the increase in the number of neutrophils whose content 2 and 4 hours following exposure exceeded the original by 100 and 36%, respectively. At the same time, however, we observed a leftward shift due to an increase in the stabform

TABLE 1. CHANGE IN PERIPHERAL BLOOD INDICES IN IRRADIATED DOGS*

Time after irradiation, days	Leucocytes, thousands	Neutrophils, thousands	Lymphocytes, thousands	Eosinophils, thousands	Thrombocytes, thousands	Time after irradiation, days	Leucocytes, thousands	Neutrophils, thousands	Lymphocytes, thousands	Eosinophils, thousands	Thrombocytes, thousands
	Dose, 100 r (7 dogs)						Dose, 200 r (4 dogs)				
Background	13,4	7,6	2,65	0,86	227,0	Background	10,7	6,42	2,6	1,0	343,8
2 hrs	13,9	8,4	2,62	0,69	215,7	2 hrs	11,72	7,9	2,2	0,9	389,3
4 hrs	12,4	7,86	2,3	0,35	308,0	4 hrs	9,86	7,05	1,54	0,76	265,8
1	10,9	5,66	1,7	0,71	300,0	1	5,86	4,2	0,71	0,54	444,5
3	9,3	5,86	1,2	0,97	244,1	3	75,87	4,8	1,14	0,74	231,2
7	7,7	5,1	1,0	0,74	221,5	7	60,2	3,24	1,06	0,86	356,2
10	8,08	5,55	1,19	0,77	130,0	10	55,7	2,67	0,95	0,59	101,0
15	8,5	7,2	1,39	0,42	136,0	15	3,98	3,89	1,05	0,1	78,0
20	7,7	5,0	1,2	0,46	220,0	20	3,88	3,48	1,15	0,22	51,1
25	8,9	6,77	1,56	0,6	248,4	25	6,6	3,48	1,39	0,39	129,4
30	9,6	6,59	2,2	0,6	336,7	30	7,4	5,1	1,55	0,67	193,6

*Commas represent decimal points.

neutrophils. At later stages of observation, changes in the absolute quantity of neutrophils also reflected the dynamics of the fluctuations of the total number of leucocytes.

Characteristic features of the reaction at the early stages of observation were lymphopenia, eosinopenia and thrombocytopenia (Figures 2, 3). Thus, 2 and 4 hours following irradiation the number of lymphocytes was 52 and 33% (respectively) of the original level, while without vibration the number of these cells decreased significantly. The difference amounted to 46, 53 and by the end of the first day — 22%. During subsequent periods, the

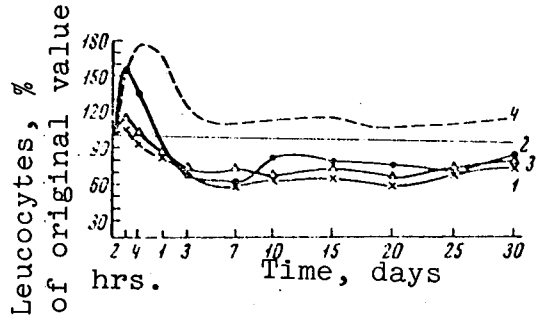

Figure 1. Change in number of leucocytes with the combined action of vibration and irradiation at a dose of 100 r:
1 — irradiation; 2 — vibration 2 hours before irradiation; 3 — vibration 1 day before irradiation; 4 — vibration

TABLE 2. CHANGE IN PERIPHERAL BLOOD INDICES IN SIX DOGS, IRRADIATED AT A DOSE OF 100 r WITH PRELIMINARY ACTION OF VIBRATION (2 HOUR INTERVAL)*

Time after irradiation, days	Leucocytes, thousands	Neutrophils, thousands	Lymphocytes, thousands	Eosinophils thousands	Thrombocytes, thousands
Background	10,1	5,9	2,6	0,55	108,1
2 hrs	15,7	12,4	1,4	0,18	69,9
4 hrs	13,7	8,0	0,9	0,26	68,1
1	9,1	6,7	1,1	0,5	124,8
3	6,9	4,9	0,8	0,4	162,8
7	6,1	3,9	1,0	0,4	173,8
10	8,2	5,4	1,2	0,7	76,9
15	8,1	5,8	1,0	0,5	108,8
20	7,7	4,6	1,4	0,35	141,7
25	7,1	4,1	1,7	0,4	140,7
30	8,1	5,2	1,6	0,3	120,5

* Commas represent decimal points.

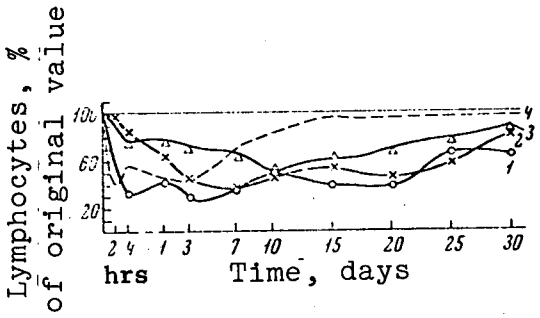

Figure 2. Change in number of lymphocytes with the combined action of vibration and irradiation at a dose of 100 r:

1 — vibration 2 hours before irradiation; 2 — irradiation; 3 - 4 — same as Figure 1.

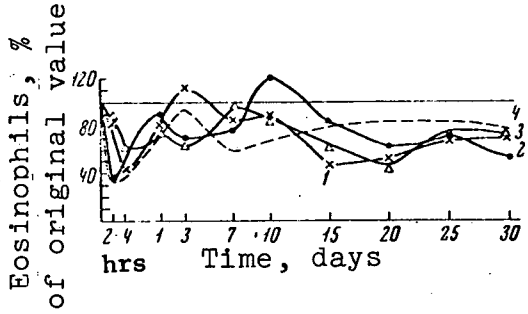

Figure 3. Change in the number of eosinophils under conditions of combined action of vibration and irradiation at a dose of 100 r:

Symbols same as in Figure 1

difference was less pronounced, but in the experimental group the quantity of lymphocytes was slightly lower than in the controls.

The number of eosinophils decreased to 33% of the original level 2 hours following irradiation. In the group with irradiation alone, the decrease in this index was insignificant. With a statistical analysis the difference turned out to be reliable

($p < 0.05$). During the remaining periods, the number of eosinophils in the experimental and control groups varied markedly.

Two and 4 hours following irradiation, we also noticed a decrease in the number of thrombocytes by 65 - 63% of the original value. Under the influence of radiation alone, the number /30
of blood platelets at these same times either did not change or increased. Further observation revealed that both the experimental and control groups showed changes in this index that were within the limits of physiological scatter (see Table 2).

In that group of animals where the interval between exposures was increased to 24 hours, the dynamics of the changes in the peripheral blood was nearly the same as in the controls (Table 3). However, as in the group with the 2-hour interval, there was a tendency toward a less pronounced decrease in the total level of leucocytes, mainly due to the lymphocyte cells (see Figure 2).

In another experiment, the radiation dose was increased to 200 r. Under these experimental conditions, the group with the 2-hour interval between exposures 2 hours after irradiation had a leucocyte count that was increased by 33% relative to the initial value (Table 4).

On comparison with the control, the difference was 23% (Figure 4). After 4 hours, the number of leucocytes dropped and nearly reached the original value. During all the remaining periods, this index was the same as in the controls.

An analysis of the morphological composition of the blood revealed that the changes in the neutrophils completely reflected the dynamics of the changes in the total number of leucocytes.

TABLE 3. CHANGE IN PERIPHERAL BLOOD INDICES OF SIX DOGS, IRRADIATED AT A DOSE OF 100 r WITH PRELIMINARY ACTION OF VIBRATION (INTERVAL OF 24 HOURS)*

Time after irradiation, days	Leucocytes, thousands	Neutrophils, thousands	Lymphocytes, thousands	Eosinophils, thousands	Thrombocytes, thousands
Background	10,6	6,4	2,1	1,1	170,8
2 hours	12,2	8,4	1,9	1,0	129,7
4 hours	10,7	7,9	1,6	0,7	141,9
1	9,2	5,8	1,6	0,8	171,6
3	7,8	4,9	1,5	0,7	—
7	7,7	4,7	1,4	1,1	153,8
10	7,2	4,7	1,0	1,0	124,0
15	7,8	5,4	1,2	0,7	128,8
20	7,0	4,6	1,4	0,5	139,5
25	7,8	4,6	1,6	0,84	125,8
30	8,5	5,1	1,8	0,77	--

*Commas represent decimal points.

The number of lymphocytes 2 hours following exposure decreased to 36% of the original level, while in the irradiated animals this index remained almost constant (Figure 5). Statistical analysis revealed that the difference was reliable.

During all the remaining observation periods, the number of lymphocytes remained at a lower level than in the radiation controls (the difference was not reliable).

Two hours following the exposures, we also noticed a drop in the number of eosinophils by 22% with respect to the original levels (in the radiation controls — by 91% of the original level) (Figure 6). Comparison with the controls revealed a

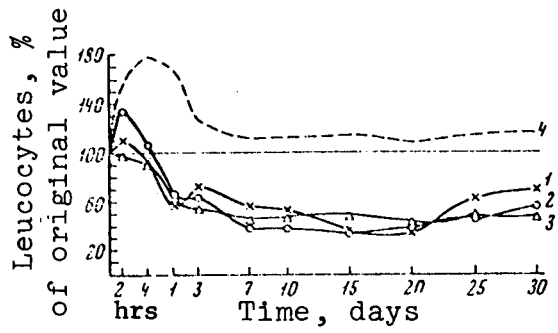

Figure 4. Change in number of leucocytes with the combined action of vibration and irradiation at a dose of 200 r:

Symbols are the same as Figure 1

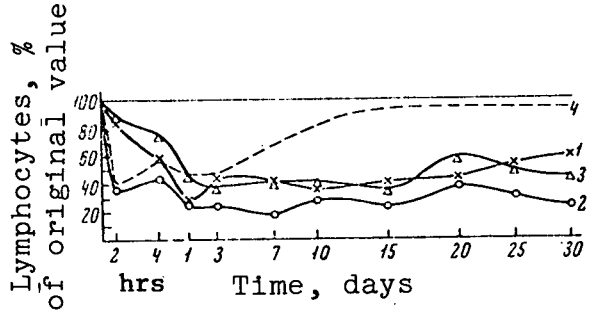

Figure 5. Change in number of lymphocytes with the combined action of vibration and irradiation at a dose of 200 r:

Symbols are the same as Figure 1

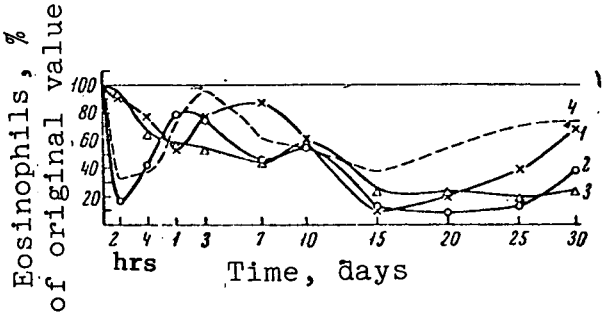

Figure 6. Change in the number of eosinophils with the combined action of vibration and irradiation at a dose of 200 r:

Symbols are the same as Figure 1

TABLE 4. CHANGE IN PERIPHERAL BLOOD INDICES IN THREE DOGS, IRRADIATED AT A DOSE OF 200 r WITH PERIPHERAL ACTION OF VIBRATION (INTERVAL OF 2 HOURS)*

Time after irradiation, days	Leucocytes, thousands	Neutrophils, thousands	Lymphocytes, thousands	Eosinophils, thousands	Thrombocytes, thousands
Background	14,1	5,7	6,2	1,3	342,7
2 hours	18,8	15,1	2,24	0,29	108,3
4 hours	14,9	10,5	2,66	0,53	96,4
1	8,9	5,3	1,55	1,02	283,8
3	8,6	5,2	1,53	0,96	121,3
7	5,1	3,1	0,96	0,58	--
10	5,5	2,6	1,56	0,74	94,4
15	4,5	2,8	1,26	0,13	48,5
20	5,33	3,4	2,2	0,11	126,5
25	6,4	3,7	2,1	0,14	155,9
30	7,7	4,7	1,48	0,48	208,2

*Commas represent decimal points.

statistically reliable difference. During subsequent periods, both the experimental and control groups failed to show any pronounced and regular changes.

After 2 and 4 hours, we found pronounced thrombocytopenia in this group (in contrast to the controls). By the end of the first day, the number of thrombocytes had almost returned to the original level, but beginning with the third day we again noticed a drop in this index. In the irradiated animals, a decrease in the number of blood platelets was recorded beginning with the 10th day of observation. The process of recovery in both groups began on the 25th day, but toward the end of the observation

period this index in both the experimental and control groups had not reached the original level.

In the group of animals where the interval between exposure was increased to 24 hours, the dynamics of the changes in the peripheral blood was nearly the same as in the radiation controls /31 (Table 5). However, analyzing the data from the early stages of observation, we can state that the reaction to radiation in the experimental group differed slightly from that in the controls. In the experimental group, only 2 hours after exposure, the drop in the number of leucocytes began, while in the radiation controls

TABLE 5. CHANGE IN INDICES OF PERIPHERAL BLOOD IN THREE DOGS IRRADIATED AT A DOSE OF 200 r WITH PRELIMINARY ACTION OF VIBRATION (INTERVAL OF 1 DAY)*

Time after irradiation, days	Leucocytes, thousands	Neutrophils, thousands	Lymphocytes, thousands	Eosinophils, thousands	Thrombocytes, thousands
Background	12,7	6,11	2,75	2,1	206,1
2 hours	12,3	7,1	2,39	1,99	9,40
4 hours	11,6	7,1	2,1	1,4	204,8
1	8,1	5,1	1,22	1,2	—
3	6,88	4,46	0,99	1,14	268,7
7	5,73	3,48	1,06	0,87	—
10	5,85	2,9	4,0	1,26	83,9
15	5,9	3,6	1,4	0,47	—
20	5,3	2,9	1,55	0,45	75,0
25	5,8	3,5	1,3	0,41	115,6
30	6,2	4,18	1,15	0,49	159,2

*Commas represent decimal points.

we recorded a typical reaction to the radiation which was characterized by a slight increase in the number of leucocytes with a subsequent decrease in their number (Figure 4). Beginning with the first day, the dynamics of the changes in the peripheral blood in the experimental group was the same as in the controls. An analysis of the morphological composition of the blood failed to show any important differences between experimental and control groups. The dynamics of the changes in the blood platelets was nearly the same as in the controls. The difference was observed only 2 hours following the exposure (in the experimental group, 40% vs. 115% in the controls).

As far as the red blood indices are concerned, they were within the limits of physiological scatter in all groups. We also failed to observe any important differences in the weight and clinical state of the experiment and control animals.

Hence, when comparing the dynamics of the changes in the peripheral blood indices, with joint exposure to vibration and penetrating radiation, with changes in these indices that arise under the influence of each of these factors separately, we must point out that their nature depends on the interval between exposures and the radiation dose. This dependence was most clearly revealed in the first days following irradiation.

Thus, in the group with a 2-hour interval between exposures, where the radiation dose was 100 r, the changes that were recorded during the first days were caused primarily by the influence of vibration.

It is evident from Figure 7 that a vibration alone in the course of the first days produced pronounced changes in the peripheral blood. Immediately after its action, however, we

observed a gradually increasing rise in the number of leucocytes, reaching a maximum level 4 hours after the end of the vibration (176% of the original level). At the same time, however, the observations revealed neutrophilia, lymphopenia, eosinopenia, and a decrease in the number of thrombocytes. Against this background there was also an effect of radiation. At a dose of 100 r, it was a weaker stressor than vibration. With their joint action, we could expect the effects caused by each factor to be combined. However, this did not take place. The radiation at a dose of 100 r reduced the effect of the vibration with respect to practically all investigated indices, and the result in the complex action group was found to be less than in the group with vibration alone, and higher than in the group with radiation alone. Radiation at a dose of 200 r decreased the effect of vibration on the total number of leucocytes (neutrophils) to a greater degree than at a dose of 100 r. At the same time, however, judging by a number of indices (lymphocytes, eosinophils) the effects from the two stressors were combined, in which the leading factor continued to be the vibration. At later stages of observation, the differences between the group with the complex effect and the groups in which vibration or radiation alone were used were less pronounced than during the first few days. However, the level of the total number of leucocytes in the group with the

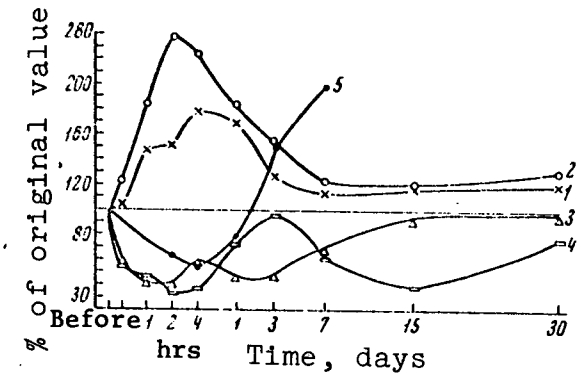

Figure 7. Change in peripheral blood under influence of vibration:

1 — leucocytes; 2 — neutrophils; 3 — lymphocytes; 4 — eosinophils; 5 — thrombocytes

/31

complex exposure was higher, and the level of the number of lymphocytes was lower than with radiation alone. Consequently, we can suggest that lymphoid tissue is more sensitive to the action of a dynamic factor, which obviously changes its functional state considerably and is expressed in the nature of the reaction to the radiation.

A completely different picture was observed with an increase in the interval between exposures of up to 24 hours. Thus, during the first 24 hours following irradiation in the group with complex exposure there was no pronounced leucocytosis of the neutrophilic type, as was the case with the 2-hour interval. At subsequent observation times, instead of a decrease in the number of lymphocytes (interval of 2 hours), the combined group showed an increase relative to the group where radiation alone was effective. Probably this difference can be explained by the fact that with the 2-hour and 24-hour intervals the radiation acted against a different background, created by vibration. In the first case, the action of the radiation occurred during the period of pronounced changes affecting various indices in the peripheral blood, produced by vibration, while in the second it was at a period when these changes had not yet become evident.

Carrying out an analysis of the changes in peripheral blood, we must not fail to consider other changes that arise under the influence of vibration. In particular, it was shown that vibration produces disruption of the division of the marrow cells [1, 2], reduces the mitotic activity of the marrow and spleen cells [2, 4], causes depression of oxidation processes [5, 7], and lowers blood serotonin [10]. These changes characterize the changes in the reactivity of the individual systems of the organism, including hemopoiesis; consequently, this must have some influence on the final effect of the radiation.

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Influence of Preliminary Administration of the Radioprotector "Finam" on the Reactivity of the Tissues of Irradiated Animals Under Various Experimental Conditions

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ABSTRACT. The author studied the influence of the radioprotector "finam" on the post-radiation changes in a number of internal organs in experimental animals following single and double gamma irradiation, as well as following combined exposure to the effect of gravitational overload and radiation. It was

found that the use of the protector reduces the degree of post-radiational changes and promotes a more rapid and complete recovery of the damaged tissue.

This paper is devoted to a study of the role of the radio- /314
protector "finam" which belongs to the aminothiols and reduces the degree of morphological change in a number of the internal organs of irradiated animals. We analyzed the influence of the radioprotector on the tissue structure of the myocardium during the first 1.5 months of radiation sickness, the course of the regenerative process in the sciatic nerve of the irradiated animals, the processes of recovery and morphological adjustment in various tissues and organs at remote periods following double gamma irradiation, and also following combined exposure to overloads and radiation.

The material from the experimental animals was fixed in a 10% solution of neutral formalin in Carnot and Shabadash fixers, then cast in celloidin, paraffin and in paraffin-celloidin. From a portion of the non-fixed material, we prepared fresh-frozen sections. The preparations were stained with hematoxylin, eosin, picrofuchsin according to Mallory, Selye, Weigert and Nissl, with gallocyanin. Histochemical methods were used for the lipids, glycogen, neutral and acid mupolysaccharides, NH_2 and SH-groups, succinodehydrogenase, cytochromoxidase and acid phosphatase. For a quantitative estimate of the damaging effect of the radiation and the role of the radioprotector in the change in reactivity, we used the karyometric method* and

*Editor's Note: This is measurement of a cell nucleus.

photometry of myocardium preparations, processed for NH_2 groups and the enzymes of the succinoxydase system.

Influence of Preliminary Administration of "Finam" on the Development of Radiation Damage in the Myocardium with Single Gamma Irradiation at a Dose of 1000 r

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In the experiments, we used 170 white rats with an original weight of 150 - 210 g. The animals were irradiated on the GUT-Cobalt-400 gamma apparatus at a dose of 1000 r. The radioprotector was administered intraperitoneally 10 minutes prior to irradiation at the rate of 200 mg/kg. The animals were killed with ether vapor 15 - 20 minutes, 1.3 - 4, 7 - 8, 10 - 11, 14 - 15, 20, 30, and 45 days after the exposure.

During the first observation period (15 - 20 minutes following the end of irradiation), we did not find any differences in the changes in the myocardium in the animals that did and did not receive finam. During all the other periods, and especially during the height of the radiation sickness (7 - 15 days), the severity of the morphological changes was less in the white rats which received the radioprotector prior to irradiation. This could be followed with the aid of all of the histological methods used in this study.

When the non-fixed material processed according to Selye was analyzed, it was found in the combined blocks that the severity and extent of the fuchsinophilia in the myocardium of the animals irradiated with chemical protection was less than in those that had been simply irradiated, although it was slightly higher than in the intact animals (Figure 1). In the protected

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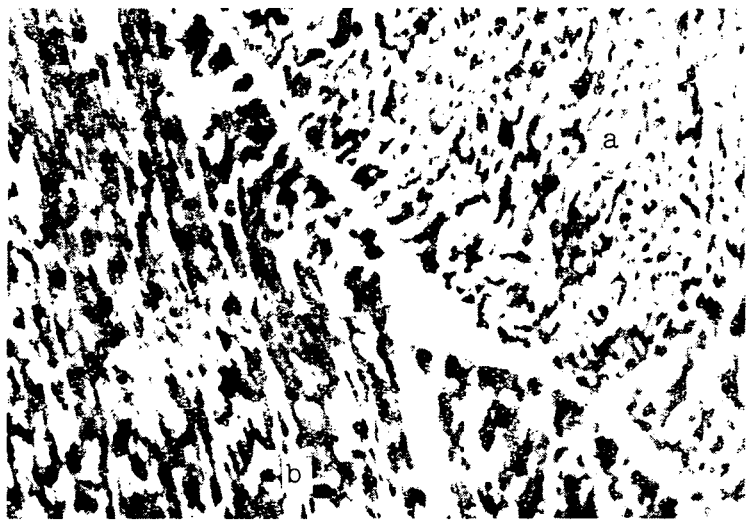

Figure 1. Fuchsinophilia of myocardium fibers of white rat which received finam prior to gamma irradiation at a dose of 1000 r (a) as compared to simply irradiated animals (b)

Stained according to Selye, unfixed sections of combined block. Objective 10, ocular, 15

white rats, we found a more intensive reaction to amine groups on the first and fourth days. To a lesser degree we found muscle fibers with sharply increased reaction to NH_2 and SH-groups. The phenomena of lipophanerosis, disturbance of hyperemia, calcium deposition, changes in the nature of the secondary luminescence of muscle fibers and other morphological symptoms of radiation damage to the myocardium were observed more rarely in these animals at all stages of the investigation.

The use of finam prior to irradiation promoted lesser changes in the activity of the enzymes of the succinoxydase system. The increase in the activity of the investigated enzymes

during the first day (Figure 2) and its decrease during the height of the disease in the protected animals was much less evident than in the unprotected animals.

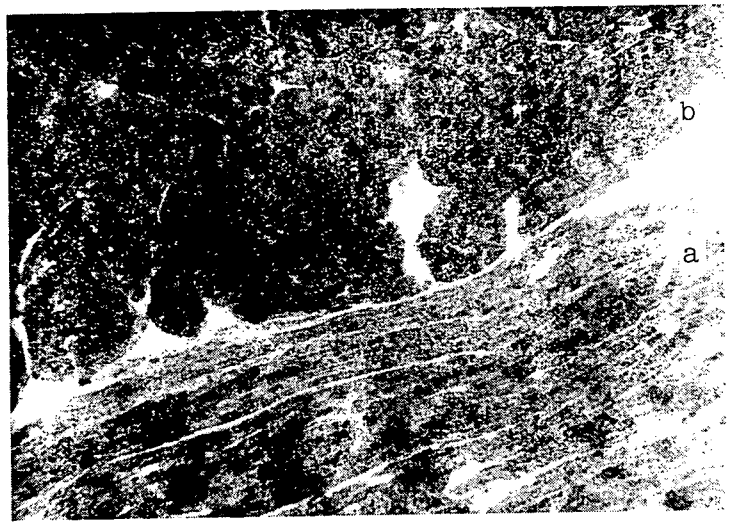

Figure 2. Activity of succinodehydrogenase in the myocardium of the white rat which received finam at a dose of 1000 r prior to irradiation (a), as compared to unprotected animal (b). First days of experiment.

Method with NST*. Objective 10, ocular 7

One to one and a half months following gamma irradiation at a dose of 1000 r, the difference between the animals of the compared series consisted primarily in the quantity and distribution of muscle fibers with altered tinctorial properties. The phenomena of fuchsinophilia and orangephilia of the myoplasm and increased sorptive capacity with respect to acridine orange, as well as increase in the reaction NH_2 and SH groups, was observed in a smaller number of muscle elements.

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*Translator's Note: Expansion not available.

The most pronounced protective effect of preliminary administration of finam — using as an example attenuation of postradiation damage to the myocardium of white rats, irradiated at a dose of 1000 r — was detected by means of photometry. /317

In measuring the intensity of the light transmitted by myocardium preparations of the series being prepared and the controls, we found statistically reliable data that support the visual observations of the small changes in the quantity of NH_2 groups and the activity of the enzymes in the succinoxydase system in the protected animals (Table 1, Figure 3). The evaluation of the quantitative changes was performed both with sections from a given combined block and by calculation of the average index of optical density ($\Delta D_x = \log \frac{I_{control}}{I_{exp}}$, where $I_{control}$ is the intensity of the light transmitted by the control section and I_{exp} is the experimental value).

Development of Post-Traumatic Regeneration of the Sciatic Nerve in Animals Irradiated Under Conditions of Chemical Antiradiation Protection

In these experiments, we used 150 white rats weighing 150 - 180 g. The animals were irradiated with x-rays on the RUM-3 apparatus at a dose of 800 - 1000 r and on the GUT-cobalt-400 gamma-apparatus at a dose of 1000 r. Ten minutes before irradiation, some of the animals received finam in the form of a 10% aqueous solution administered intraperitoneally at the rate of 125 mg/kg. The left sciatic nerve was traumatized in all the animals on the 7th day following irradiation by applying a

TABLE 1. EFFECT OF PRELIMINARY ADMINISTRATION OF FINAM ON THE INDEX OF OPTICAL DENSITY, REFLECTING CHANGES IN THE NUMBER OF FREE NH_2 GROUPS AND THE ACTIVITY OF SUCCINODEHYDROGENASE IN THE MYOCARDIUM OF WHITE RATS ON THE 1 - 7th DAYS AFTER IRRADIATION AT A DOSE OF 1000 r.

Period of investigation, days	Group	NH_2 groups (M ± m)	p <	Succinate dehydrogenase (M ± m)	p
1	Irradiation	1.6919±0.0024	<0.001	0.0659±0.0013	<0.005
	Finam + irradiation	1.8686±0.005		0.0376±0.0038	
4	Irradiation	1.7421±0.0057	<0.001	–	
	Finam + irradiation	1.8823±0.0087		–	
7	Irradiation	1.1264±0.0086	<0.001	1.9591±0.0014	<0.001
	Finam + irradiation	0.0030±0.011		1.9855±0.0013	

Holstead clamp to the upper third of the femur. The animals were killed on the 1st, 3rd, 5th, 7th, 9th - 10th, 15th, 20th, 30th, 60th and 90th days and 5 months following traumatization of the nerve. We studied the left sciatic nerve, the left gastrocnemius muscle, and the corresponding spinal ganglia.

The study of the material obtained from the irradiated animals and its comparison with the control data revealed that /318

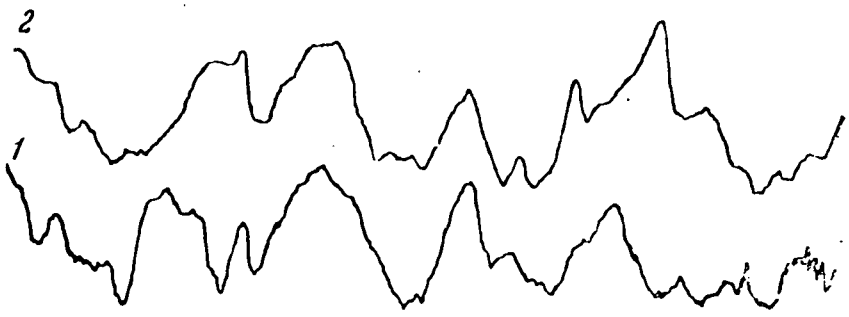

Figure 3. Photometric curves reflecting differences in the amount of free, reactive amine groups in the myocardium of white rats which did (1) and did not (2) receive finam prior to irradiation at a dose of 1000 r. 24 hours after the experiment. Recorded on electronic potentiometer directly from photomultiplier

the recovery of the nerve in the irradiated animals proceeds with the same characteristics as in the controls. However, a number of the characteristics that developed in the form of a slowdown in the recovery period, a reduction of the resorption of the breakdown products, and destruction of the newly formed elements, was found in all the animals with severe radiation syndrome.

By the 20th day, the intact animals began to show active neurotization of the intramuscular trunks and muscle fibers. In the irradiated animals, the entire length of the peripheral segment showed a large amount of remaining decay products. There were very few axons in the trunks. The formation of the terminal apparatus was not observed. By the 30th day, we observed active formation of terminals in the non-irradiated animals and only the beginning formation of terminal branches in the non-irradiated animals. At later stages (45 - 60 days) the animals that had survived radiation sickness had a very primitive structure of the terminal apparatus.

In those animals which had received a radioprotector for prophylactic reasons, the radiation sickness had a much milder course. The resorptive-recovery processes were more pronounced. The picture of post-traumatic regeneration in these animals resembled that in the controls (non-irradiated animals). /319

With degenerative processes in the nerve fibers and wallerian degeneration, the Swann cells show clear reactive changes that are manifested particularly in their increased proliferation and an associated change in the volume of the nuclei [8]. These features can be employed to determine the degree of activation of the peripheral glia and the course of the degenerative process in the nerve. We used the method of calculating the density of Swann cells characterizing the degree of their proliferation and karyometry as an index of their functional state. At each stage in the compared series we collected preparations from six animals for analysis by quantitative methods.

On the 5th day following the trauma, we failed to observe any significant differences in the cell density per unit area in the control and experimental animals. Nine days after the trauma, the density of the nuclei of the peripheral glia in the irradiated animals was significantly less than in the controls. In those animals which had previously received the radioprotector, the average number of cells approached that in the controls. By the end of the second week after the trauma, this delay in proliferation of the peripheral glia became still more pronounced. The density of the nuclei per unit area in the irradiated animals at this time was nearly 1.5 times less than in the controls. In those animals which were irradiated with the radioprotective preparation, the nuclei density of the Swann cells approached that of the controls. A similar pattern, but with less difference, was retained on the 20th and 30th days after trauma. In

those animals which had previously received a protector, the density of the nuclei of the Swann cells even at this time was very similar to the controls.

As revealed by a karyometric study, the control animals in the process of regeneration undergo a definite increase in the nuclei of the peripheral glia, attaining a maximum toward the end of the second week. While 5 days after the trauma the nuclei with log V < 2.0 (40.9%) were predominant, by the 15th day nuclei with log > 2.0 (54.75%) were predominant. Beginning with the 20th day, the volumes of the nuclei begin to return to the original state and by the 30th day are nearly normal.

In the irradiated animals, this feature was retained on the whole, but the entire histogram is lower and displaced further to the right than for the controls. It is particularly this difference that was evident in all three series on the 5th day. While in the control animals the nuclei with a volume log V > 2.4 amounted to 2.26%, in those that were irradiated without protection it was 9% and 5.52% for those receiving finam. The maximum volume of the nuclei in the controls amounted to log V = 2.6, with irradiation of log V = 2.9, and in the animals that were irradiated against a background of preliminary administration of finam — log V = 2.8.

Hence, irradiation leads to definite changes in the peripheral glia, taking the form of a delay in proliferation and an increase in the volumes of the nuclei. At the same time, the experimental animals showed a lag in the regeneration process of the peripheral nerve of the rat. The prophylactic administration of finam to rats produces this effect, causing a certain stimulation of the process taking the form of a more intensive

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proliferation of the glia (by the 20th day) than in the controls, and an acceleration of the regeneration process.

Influence of Finam on Remote Post-Radiational Changes in the Internal Organs Following Double Gamma Irradiation

The influence of single and double administration of finam with fractional irradiation on the degree of remote morphological changes was studied on white mice with an original weight of 18 - 20 grams. The animals were irradiated twice using the "Khizotron" gamma apparatus at a dose of 12.5 - 15.5 r/min. The interval between irradiations was 49 days. The first radiation dose consisted of 100, 300, 500, 700 or 900 r, while the second dose was 900 r. The protector was administered intraperitoneally at the rate of 200 mg/kg 10 to 15 minutes before each (or only before the second) irradiation. The animals were killed by ether vapor 6 - 7 months after the second exposure. As a control we used material from 6 intact mice that were kept under similar conditions during the experiment. For morphological study, we used 139 animals which were divided into 3 series.

In the first series, the protector was not given. In the second, finam was given only before the second irradiation, and in the third group it was given before each exposure to radiation. At the time the experimental animals were killed, of the first group that had received the first dose of radiation of 100 - 300 r about 30% were alive; 20 - 25% of those that received 500 - 700 r still remained, and all had died that received the 900 r dose. In the second series, with an initial irradiation dose of 100 r, 75% were alive; 300 r — 50%; 500 r — 30%; 700 r — 20%; and at a dose of 900 r, all had died. When finam was

administered prior to each irradiation, the dose required was much higher; thus, with an initial radiation dose of 700 r 75% survived, while 50% survived at 900 r.

When the experimental animals were autopsied (interval between irradiations equals 49 days), the external appearance as a rule was not unusual. It is true that in the first series we quite often found non-uniform plethora of the rear (lower) extremities of the lungs and liver. The apex of the heart in these animals was rounded, the heart muscle was flaccid, and the right atrium was slightly distended. In some mice we found tumors of the femur (osteochondrosarcomas). In the second series, with an initial irradiation dose of 700 r, we also found signs of non-uniform plethora of the lungs and liver, and changes in the configuration of the heart.

A histological examination of the marrow in the spleen /32
which was performed on preparations stained with hematoxylin-eosin and picrofuchsin according to Pappenheim and processed according to Feulgen for DNA, we did not find any noticeable differences at all in the series which were compared. In the surviving animals, even after exposure to radiation at an initial dose of 700 r and a second of 900 r with an interval of 49 days between (especially in the 3rd series), we found no signs of radiation damage or inhibition of hemopoiesis after 216 days. The structure of the marrow in the spleen (Figure 4) differed in almost no respect from that in the control material. To be sure, the only sign of former radiation damage was a slightly more coarse gialinosis of the central arterioles of the follicular apparatus of the spleen, as well as a slight thickening of the capsula and trabeculae. The absence of changes in the marrow and spleen has to do with a very high proliferative activity and rapid recovery of the cellular substance.

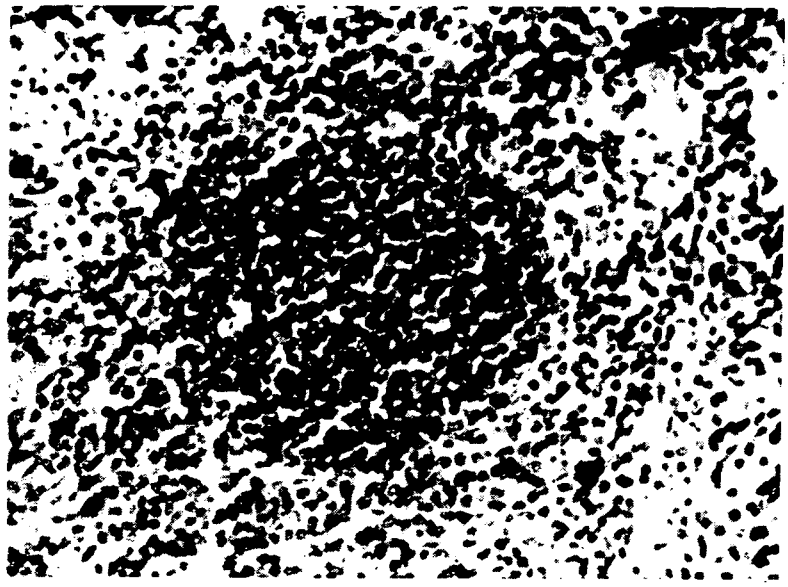

Figure 4. Spleen of white mouse 216 days after double (with 49 day interval) irradiation with gamma rays in doses of 700 and 900 r. Prior to each exposure the animals received finam. A Malpighian body was formed.

Photomicrograph. Objective 10, ocular 10

A study of the morphological changes in the duodenal intestine gave some very sparse data on the remote consequences of double irradiation and the prophylactic use of finam to attenuate radiation damage. The influence of the prophylactic /322
effect of finam on the radiation damage to the duodenal intestine was more clearly evidenced in a comparison of the average number of cells in one crypt, the mitotic index and the percentage of cells with chromosomal aberrations relative to the entire examined figure of mitosis. In the control, the average number of cells per crypt was 71.4 $\pm$ 0.4. In the animals with a dose of initial radiation of 100 r in the first series, there were 77.4 $\pm$ 0.9, while in the third at the level of the control there were

(71.4 ± 1.0). At a dose of 700 r in the first series, it was 80.1 ± 0.9, in the second 76.9 ± 0.8, and 74.0 ± 0.6 in the third. The validity of the difference between the data of the controls and the compared series was within the limits of $p < 0.02 - 0.5$

The irradiation aftereffects took the form of changes in the mitotic index, so that at the first irradiation doses of 100 and 300 r we observed an increase in it (69.75 ± 0.75 and 50.0 ± 1.0), while at doses of 500 and 700 r there was a slight decrease to 39.3 ± 0.3 and 42.0 ± 1.0 (44.1 ± 1.0 in the controls). This apparently is associated with the fact that, at small doses of initial irradiation, recovery proceeds at more rapid rates, primarily due to division of epithelial cells. As support for this position, we have the considerable growth of connective tissue elements at an initial irradiation dose equal to 500 and 700 r. Consequently, in these cases the recuperative processes in the duodenal intestine are governed to a large extent by the processes of compensating regeneration.

The use of the radioprotector prior to radiation reduces the degree of damage of the mucous membrane, clearly indicated by the changes in the mitotic index. Thus, while at an initial irradiation dose of 100 r the mitotic index in the first series was 69.75 ± 0.75; in the third series it was 38.0 ± 1.5. At a dose of 300 r, in the first series it was 50.5 ± 1.0, in the second series — 38.5 ± 0. In the third series, it was 47.3 + 1.75. While we observed its decrease to 39.9 ± 0.5 with an initial irradiation dose of 500 r, the double use of finam promoted its increase even by comparison to the control (64.0 ± 3.5). In the series with an initial irradiation dose of 700 r, single and double administration of finam did not have a noticeable effect on the mitotic index.

In comparing the percentage of cells with chromosomal aberrations only in those animals whose initial dose of irradiation was 100 or 500 r, we noticed considerable differences both in the control data and between the series that were compared. In the first series at a dose of 100 r, the percentage of chromosomal aberrations was 5.9 ± 0.5, while in the third series it was 10.4 ± 0.9. At a dose of 500 r we observed the opposite effect: in the first series we had 10.9 ± 0.6 and in the third series — 5.9 ± 0.3 (6.5 ± 0.3% in the control). We could try to explain this by the fact that at small doses there is a very intensive /323 recovery accompanied by a sharp rise in the mitotic activity. The genetic damage is not so great, and therefore the aberrant forms occur among a large mass of normal mitoses. The action of a chemical agent prior to the second irradiation, leading to hypoxia phenomena in the organism, is an additional factor which determines the development of genetic damage. Inasmuch as in this situation we also observe protection against destructive postradiational changes, recovery takes place more rapidly and it has already stopped at the time the animals are killed. This is indicated by the low mitotic index (38.0 ± 1.5). In this connection, a slight increase in the absolute number of cells with chromosomal damage gives a high percentage of aberrations.

At high doses (500 and 900 r) recovery proceeds much more slowly, the mitotic index is low (39.3 ± 0.3), and the tissue damage is more severe, so that it leads to a large percentage of chromosomal aberrations. Administration of finam reduces the damage and suppression of compensatory mechanisms. Therefore, restoration proceeds more rapidly. This is characterized by the higher mitotic activity which, as we have already mentioned earlier, decreases the percentage of aberrations even if their total number is high.

In all the other series, there were no significant differences. In those animals whose initial radiation dose was 300 r, this in all probability is related to the fact that they occupy an intermediate position between the series that are characterized by high (100 + 900 r) and, on the other hand, low mitotic indices (500 + 900 r). In the experiments with an initial irradiation dose of 700 r, we can explain the nearly equal percentages of chromosomal aberrations and approximately equal mitotic indices in the compared series by the predominance in postradiation recovery of the mucous duodenal intestine or processes of restorative regeneration.

Hence, the prophylactic use of finam not only reduces irradiation damage to the intestine, as was repeatedly observed in experiments using various radioprotective preparations [14, 19, 21], but it also promotes a more complete recovery at remote periods.

Reliable data on the role of the protector in the normalization of metabolic processes and the functional state at remote times were obtained by a karyometric investigation of the liver. For this purpose, we measured the volumes of the nuclei of the hepatic cells in a series of animals with initial radiation doses equal to 500 and 700 r. Following the change in the volumes of the nuclei and their logarithmization, two classes were formed containing most of the nuclei. The influence of penetrating radiation and prophylactic administration of finam was evaluated on the basis of the changes in the percentile content of the nuclei in these classes. We also computed the logarithmic and geometric averages, reflecting the changes in the average /32
volumes of the nuclei for the various series of the experiment.

In the control, the nuclei of the hepatic cells were distributed as follows: class with volume 65 - 250 microns^3 — 23.5%; with a volume of 251 - 800 microns^3 — 76.3%; and nuclei with a volume greater than 801 microns^3 — 0.2%. The logarithmic average was 2.4578 and the geometric average was 286.9 microns^3. Irradiation promoted the development of smaller nuclei and an increase in the percentile content of nuclei with volumes of 65 to 250 microns^3 at a total dose of 1400 r up to 47.1% and with a total dose of 1600 r up to 40.8%, with a decrease of the logarithmic averages, respectively, to 2.3795 and 2.3758, and the geometric averages — to 239.9 and 237.5 microns^3 (Table 2). The prophylactic administration of finam prior to the second irradiation led to a slight decrease in the average volumes of the nuclei (up to 245.5 microns^3 with a total dose of 1400 r, 239.4 microns^3 at a dose of 1600 r). The normalization of the nuclear dimensions was most clearly evidenced with a double application of radioprotector (Table 2).

The study of the aftereffects in the organ of vision showed that ionizing radiation leads to the development of marked changes that are observed 6 - 7 months after exposure. In 21 /32
animals out of 108 that were subjected to irradiation, we found cataracts. Clouding of the lens was observed primarily in those animals which had received a total radiation dose equal to 1400 - 1800 r. In 20 surviving animals which had been irradiated without protection, a cataract was found in 5; when finam was used prior to the second exposure to gamma rays, 3 animals out of 30 that survived showed cataracts before they were killed. In the series with double administration of finam, a cataract occurred in 13 mice out of 58. The latter is unquestionably related to the fact that finam promoted a sharp increase in the

TABLE 2. PERCENTILE CONTENT OF NUCLEI OF HEPATIC CELLS WITH DIFFERENT VOLUME, LOGARITHMIC MEAN AND GEOMETRIC MEAN IN A SERIES OF EXPERIMENTS WITH DOUBLE GAMMA IRRADIATION AND PROPHYLACTIC ADMINISTRATION OF FINAM

Class of nuclei	Control	First series	Second series	Third series	First series	Second series	Third series
		500 + 900 r			700 + 900 r		
Class 1, volume < 65 μ^3, log v < 1.8	–	0.6	0.6	0.4	1.1	0.6	0.6
Class 2, volume 65 - 250 μ^3, log v 1.8 - 2.3	23.5	47.1	42.4	31.5	40.8	45.2	25.1
Class 3, volume 251 - 800 μ^3, log v 2.4 - 2.9	76.3	51.2	57.0	67.7	56.7	53.0	73.7
Class 4, volume > 801 μ^3, log v > 2.9	0.2	1.5	–	0.4	1.4	1.2	0.6
Logarithmic mean	2.4578	2.3795	2.3902	2.4102	2.3758	2.3791	2.4291
Geometric mean (in μ^3)	286.9	239.6	245.5	257.1	237.5	239.4	268.6

survival rate with initial radiation doses of 500, 700 and 900 r. A fact that is worthy of attention is the lesser degree of clouding of the lens that was noted in all animals that received finam prior to each irradiation, in comparison with those that were irradiated without chemical protection.

Irradiation causes significant changes in the neurons of the retina, evidenced by a considerable increase in the size of the nuclei. While in the controls the basic number of nuclei (68.25%) had a volume of 70 - 150 μ^3, double irradiation with a total dose of 1200 r leads to a decrease in this percentage to 8.5% and an increase in the percentage of nuclei with a volume of 150 - 190 μ^3 to 39.0% and nuclei with a volume greater than 190 μ^3 to 26.5% (in the controls, nuclei with a volume of 150 - 190 μ^3 numbered 1.25%, while nuclei with a volume greater than 190 μ^3 were not found at all). Similar changes in the distribution of nuclei by volume occurred at an initial irradiation dose of 500 r. Single and double administration of finam did not significantly change the postradiation changes as far as increasing the average volumes of the neutrons of the gangliar layer of the eyeball retina were concerned. The normalizing effect of finam was evidenced only in the series with an initial irradiation dose of 100 r, so that even a single administration of a radioprotector promoted almost complete recovery of the usual distribution of nuclei by volume and their percentile content.

A histological analysis of the changes in the brain and peripheral nervous system, following total double irradiation with an interval of 49 days without the use of a protector, showed a dependence of the damage of the tissue elements on the first dose of irradiation. Thus, with double exposure at total doses of 1000 and 1200 r (according to the systems 100 + 900 r

and 300 + 900 r), the nerve cells of the cerebral hemisphere did not undergo any physical morphological changes. With an increase in the dose of initial radiation up to 500 and 700 r, the morphological disturbances increased in severity. Many neurons of the V layer of the cortex and subcortical nuclei acquired uneven, wrinkled contours with a decrease in the chromatophilic substance. In the II and III layers of the cortex, we found neurons with concentrated tigrolysis and hyperchromia of the cytoplasm. As far as the vessels are concerned, the reaction was more or less the same at all doses, with signs of moderate sclerosis of the vessel wall; a few small vessels were sharply dilated and overfilled with blood. Here and there we found /32
signs of perivascular edema and fine diapedetic hemorrhages.

In the series of animals which received finam prior to the second irradiation, the damage to the tissue elements of the brain was much less, and particularly evident at high doses of initial radiation. The nerve cells of the cerebral cortex did not change their mass essentially, but only a small number of cells of the V layer showed hyperchromia of the cytoplasm. In the diencephalon, mesencephalon and medulla oblongata some of the neurons were found to have loose chromatophilic substance and slight peripheral tigrolysis. As far as the vessels were concerned, we did not find any significant changes. In individual areas, we found a slight dilation of the perivascular spaces.

The protective effect of the radioprotector finam was observed also by evaluating the degree of damage to the nerve fibers and the terminal nerve apparatus. Dystrophic and hyperplastic processes in the form of swelling and increased argentophilia of the axial cylinders, fragmentation, excessive growth, proliferation of the Swann cells of the neuromuscular spindles,

and the motor plaques were much less pronounced. The double application of the protector promoted a still further decrease in the level of postradiation changes in the brain and in the elements of the peripheral nervous system.

The favorable effect of this protector on postradiation changes in the brain may also be followed with single gamma-irradiation at a dose of 1000 r. Thus, in those animals that survived up to 20 days following irradiation at a dose of 1000 r, almost all the neurons of the reticular substance of the medulla oblongata showed pronounced swelling, with signs of chromatolysis (Figure 5, A). In the cases where finam was used, the majority of neurons retained their normal structure (Figure 5, B).

Microscopic investigation of the myocardium of animals of all series using ordinary extensive histological methods revealed areas of moderate homogenization of muscle fiber with disappearance of transverse striation. Here and there we found basophilia and rarely fragmentation. The nuclei of some of the fibers were large and hyperchromic, and we also found very small, lumpy ones with markedly basophilic karyoplasm. All of these phenomena were observed more or less uniformly in all the animals, and it did not appear possible to use them as a basis for evaluating the severity of the morphological changes in the myocardium in the experiments. The only criterion for evaluating the brain is the proliferation of stromal elements and the increase in the connective-tissue fibers observed to a greater degree in the first series.

The Selye method made it possible to carry out a more detailed evaluation of the protective effect of the prophylactic use of finam. In the first series, the fuchsinophilia of the myocardium was significant and involved many fibers. In the /328

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Figure 5. Reticular substance of medulla oblongata of white rat 20 days after total single exposure to gamma rays from radioactive cobalt at a dose of 1000 r:

A — giant nerve cell with eccentrically placed enlarged nucleus and deformed nucleolus; B — unchanged neurons with preliminary administration of finam

Stained with cresyl violet according to Nissl.
Ob. 40, oc. 15

myocardium of animals of the second series, fuchsinophilia of the muscle fibers was less pronounced. The layer of homogenous red-orange fibers, located beneath the epi- and endocardium, were thinner than in the animals which did not receive finam. Double application of the radioprotector promoted a still greater decrease in fuchsinophilia. We noticed a very small number of fibers with changes in the tinctorial properties on the external and internal surfaces of the heart. In the wall of the left ventricle and the interventricular septum, we found individual fuchsinophilic fibers, the majority of which retained their transverse striation.

Summarizing experiments with fractionated radiation effects against a background of chemical protection, we can conclude that the prophylactic administration of the radioprotector finam under all experimental conditions provides a high viability and reduces the severity of aftereffects evaluated on the basis of the morphological disturbances in a number of internal organs.

The Role of Finam in the Attenuation of Morphological Changes in the Internal Organs with the Combined Action of Transverse Acceleration and Penetrating Radiation

In this section, we present the results of a study on the influence of radioprotectors on the reactivity of tissue elements of internal organs under the combined influence of gravitational and ionizing factors. The experiments were performed on white female mice with an original weight of 18 - 20 grams, subjected to irradiation on the "Khizotron" apparatus with a dosė rate

of 12.5 - 15.5 r/min and the influence of transverse acceleration (chest - back) on a centrifuge with a 4.25 meter arm. Half of the animals received the radioprotector finam before the start of the experiment.

In the first group of experiments, we studied the morphological changes in the tissue elements of the brain and heart of 82 animals that were subjected to double centrifuging with an interval of 30 minutes against a background of preliminary administration of finam at 150 - 200 mg/kg. The magnitude of the overload at the time of centrifuging was 15 units. The duration of action was 5 minutes, and the rise and fall time were 30 seconds each. The animals were killed by ether vapor 2 - 157 days after the start of the experiment. Selection of organs for the first stage of investigation was governed by data in the literature concerning the great importance of disturbances in the organs of the nervous and cardiovascular systems in the disruption of normal physiological functions of the organism under /32
the influence of gravitational loads [3 - 5, 12, 17, 20, 24].

In a histological investigation of the brains of the centrifuged animals on the second day of the experiment it was found that there were numerous perivascular hemorrhages located primarily in the truncal formations. In some animals we occasionally found small foci of brain tissue destruction.

Individual cells of the V layer were swollen, with signs of breakup of chromatophilic substance. In the nuclei of the diencephalon, mesencephalon and medulla oblongata, gangliar cells with swelling and hypochromic cytoplasm were found somewhat more frequently. The distribution of ribonucleoprotein in all parts of the brain did not differ significantly from that in intact animals. On the fifth day of the experiment, the changes in the

neuronal structures became more pronounced. In the II, IV and V layers of the cortex, we always found markedly swollen gangliar cells with an increase in nuclei and signs of tigrolysis. A portion of the cells of the IV layer were wrinkled, their nuclei were deformed and hyperchromic. There were a great many dystrophically altered neurons in the nuclei of the diencephalon, mesencephalon and medulla oblongata.

In those animals that were killed on the 8th through 11th days, the changes in the neurons of the cortex were similar to those described in the preceding observation period. Subsequently, we observed a gradual normalization of the histological picture of the brain.

After 3 and 5 months, in the majority of cases there were no signs of damage that we could see. It is true that we sometimes saw signs of hypo- and hyperchromy of the neurons, vacuolization and tigrolysis of the cells of the II, IV and V layers of the cortex, pyknosis and eccentric location of the hyperchromal nuclei of the neurons of the hypothalamic region, breakup of the chromatophilic substance and changes in the distribution of ribonuclear protein in the cytoplasm in the nerve cells of the nuclei of the medulla oblongata.

Evaluating the nature and severity of the changes in the brain of animals subjected to centrifuging against the background of preliminary administration of finam, we were unable to find any characteristics that resembled those described above.

The study of the heart muscle that was performed showed that transverse acceleration leads to the development of definite morphological structural changes. During the first period of observation (2 days after centrifuging), the myocardium showed

definite signs of dyscirculatory and dystrophic processes. There was marked evidence of nonuniform venous plethora, occasionally with accumulation of slightly eosin-stained protein masses in the perivascular spaces. Individual muscle fibers were bright, with homogenous myoplasm and poorly defined transverse striation. The dimensions of the majority of nuclei were enlarged and chromatinic lumps and nucleoli were found along the long axis, with definite displacement toward one of the poles of the nucleus. The background reaction for the NH_2 group was not changed on the whole, but it is true that we did find individual fibers with sharply weakened or sharply increased reactions. When staining according to Selye, we observed pronounced fuchsinophilia of the muscle fibers in all parts of the heart. /3

In those animals which had previously received finam, the dystrophic changes and the nonuniform venous plethora were more severely pronounced. There was a more coarse fuchsinophilia, occupying a much larger area. A great many of the fibers had a pale homogenous myoplasm with unclear transverse striation. More rarely, we saw an increase in deformation of the nuclei of the muscle fibers, some of which were vacuolized, and some of which were pyknotically changed. When they were treated according to Hotchkiss, we observed considerable nonuniformity in the distribution of PAS*-positive substances in the sarcoplasm of fibers, with accumulation around individual small vessels.

After 5 - 6 days, the severity of the dystrophic processes weakened considerably. The difference in the series that were compared was not as clear as on the second day. After 11 - 15 - 21 days, the dystrophic changes were very slight. There was only

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*Editor's Note: This designates para-aminosalicylic acid.

a very small quantity of fuchsinophilic fibers. We did not observe any differences between the animals of the first and second series.

At later stages of the experiment (after 80, 86, 157 days) we noticed slight sclerotic changes in the form of thickening and growth of collagenic fibers in the subepicardial and subendocardial regions, with focused growth of connective-tissue elements between the muscle fibers and around individual small vessels. The severity of the sclerotic process, its propagation, and localization varied in different animals. We did not observe a dependence on the nature and periods of the experiment. Similar changes were also observed in certain control animals, but the frequency of their observation in the experimental animals was higher than in the intact ones.

Hence, the experiments we performed have shown that the influence exerted on the organism of experimental animals by transverse acceleration leads to the development of dyscirculatory, destructive and dystrophic processes in the brain and myocardium with gradual normalization by the 21st day. Preliminary administration of finam promotes an increase in the damage to the myocardium, which is most clearly evident in our experiments on the second day, and is less evident 5 - 6 days after the combined action. The mechanism of the modifying action in all probability is related to the inhibiting influence of finam on the metabolic processes. We know that radioprotectors from the aminothiol group promote the decrease in the oxygen tension in the tissues [13, 22, 25] and inhibit the fermentive oxidizing processes in the organoids of the cell [11, 16]. Enlargement of the mitochondria, which is observed when testing for the enzymes of the succinoxidase system in the myocardium of animals /331
15 - 20 minutes after administration of finam [15], leads us to

assume that this protector also changes the enzymatic processes. At the same time, the influence on the animal organism of gravitational overloads leads to a pronounced dyscirculatory disturbance and hypoxia of the myocardium [10]. More severe destructive changes in the myocardium of white mice under combined action are caused by intensification of metabolic disturbances that arise in response both to the administration of finam and to the action of gravitational overloads.

All of this clarifies the data found in the literature indicating the decrease in the resistance of mice and guinea pigs to the action of acceleration following administration of radioprotective preparations from the groups of the mercaptoalkylamines and indolylalkylamines [7]. The ability to withstand transverse overloads, as shown by numerous experiments, largely depends on the functional state and the compensatory capacities of the cardiovascular system [5, 18]. The administration of the protector, by intensifying the metabolic disturbances, leads to earlier exhaustion of the reserve mechanisms and a disruption of compensation, which obviously forms the basis of the decreased resistance to the gravitational effect. The fact that the study which we performed lacked a similar phenomenon in the histological study of the brain still does not prove the areactivity of the structural components of the nerve tissue to the administration of finam. In all probability, this is due to the fact that the methods employed for the morphological analysis were insufficient to reveal the fine metabolic disturbances that occur in the central nervous system under the influence of the radioprotector.

In a second group of experiments, we studied the influence of the radioprotector finam on the recovery processes in the marrow, spleen, thymus, heart muscle and brain at remote periods following the combined action of transverse acceleration and

gamma irradiation. We established three series of experiments. The first series was performed as follows: centrifuging with a gravitational overload of 15 units for a period of 5 minutes, after 50 minutes gamma irradiation at a dose of 400 r, followed by a second irradiation at doses of 550, 700 or 850 r after 15 days. The second series differed from the first in the fact that, after the first irradiation at a dose of 400 r, the white mice were again exposed to the action of transverse acceleration at an overload of 15 units for 5 minutes. In the third series, the action of the gravitational factor was excluded, and the animals were merely irradiated for the same periods of time and at the same doses. In each series of the experiment, half of the animals received finam at the rate of 150 mg/kg sixty minutes prior to the first irradiation. The study was performed 46 - 48 and 112 - 123 days following the second irradiation. We studied morphologically the material from 139 white mice that survived until the time for sacrifice.

In studying the marrow, spleen and thymus by means of the histological analytical methods we employed earlier (hematoxylin-eosin, van Gieson's solution, Pappenheim staining and Feulgen method for DNA) in all of the surviving animals, aside from the dependence on the experiment conditions, the histological picture of these organs was the same. A period of 1 - 1/2 months was completely sufficient for recovery of their normal structure (Figure 6).

The changes in the tissue elements of the brain were studied after 112 - 113 days, and we still observed slight differences in the morphological picture between the different series of experiments. The changes we found appeared to be mainly in connection with the neurons of the diencephalon, mesencephalon, and

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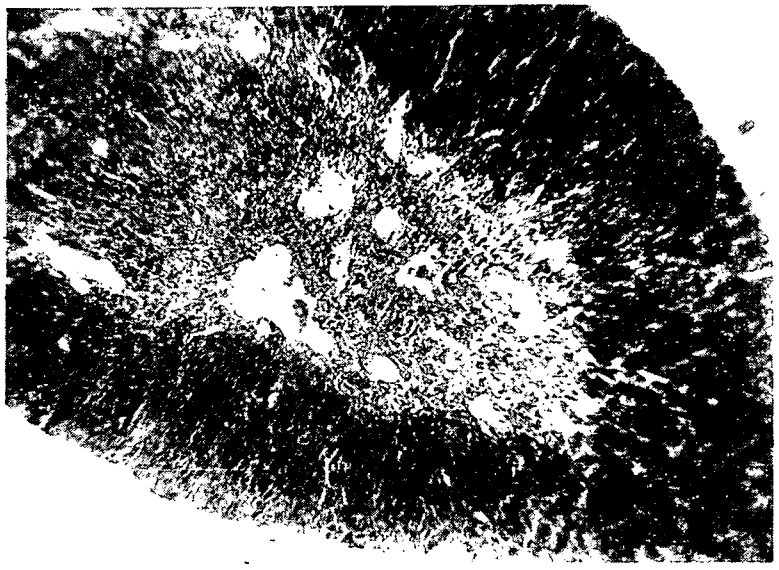

Figure 6. Thymus of white mouse 116 days following double overload (15 units, 5 minutes) and double irradiation (400 and 850 r):

Finam was given intraperitoneally prior to the first overload. Photomicrograph, Ob, 10, oc. 7

medulla oblongata. Thus, the least manifestations of the neurodystrophic process were observed in all the animals of the first series, both those which did and did not receive finam prior to the combined exposure. In the second series, a definite difference was noted depending on the administration of the radioprotector. In those animals which did not receive finam, the signs of tigrolysis, hyperchromia, swelling or vacuolization of the cytoplasm were observed in all areas, but especially sharply in the subcortical structures. In cases of preliminary administration of the radioprotector, the changes described above were /33
observed much more rarely, and only in the nuclei of the

diencephalon, mesencephalon and medulla oblongata. In the third series, we also noted the favorable effect of the radioprotector finam on the severity of the morphological disruptions that arose as a result of double gamma irradiation with an interval of 15 days.

The role of the radioprotector finam in the reactivity changes of the morphological structures under the combined action of overloads and irradiation was studied in somewhat more detail using the example of the myocardium. One of the most important characteristics of the heart muscle is its ability to react very sensitively to disruptions of the metabolic processes and to disturbances of the endocrine and nervous regulation that arise when the organism is influenced by various "stress" factors, such as gravitational overloads and radiation.

In the first series of experiments (single overload and double irradiation with an interval of 15 days), 47 days following the end of the second exposure, we observed pronounced symptoms of a dystrophic process in the form of a sharp change in the tinctorial properties of the sarcoplasm when stained according to Selye, Mallory, and iron hematoxylin. The sclerotic phenomena were pronounced mainly in the subendocardial and subepicardial areas. In comparison with the third series of experiments (double irradiation alone), the dystrophic changes were reduced. In all the animals which received finam prior to the combined treatment, the morphological disruptions were more severe, the fibers with changes in their tinctorial properties were much greater, and we noticed an irregularity in the development of NH_2 groups and neutral mucopolysaccharides. In the following period of investigation (after 112 - 114 days), there was a decrease in the dystrophic (and a slight increase in the

sclerotic) processes. We did not observe any difference due to the use of finam and the second dose of radiation.

In the second series of experiments (tests with double exposure to overloads and double irradiation), during the first period of observation we observed relatively mild severity (in comparison with the first and especially the third series) of the dystrophic processes, demonstrated by the propagation and number of the fuchsinophilic and orangephilic fibers. In those animals which received the prophylactic finam, these changes were somewhat more severe than in those which did not receive it. At the same time, however, the administration of finam' caused a significant proliferation of stromal elements with the formation of histiocytharic nodes and cords and an increase in the fibrous elements both in the thickness of the myocardium and in the endo- and epicardial portions. The difference in the morphological changes between the animals whose second radiation /3 dose was 550, 700 or 850 r was not evident.

After 115 - 123 days, the fuchsinophilia and orangephilia of the muscle fibers were more sharply pronounced and occupied larger areas than in the first period of investigation, while in those animals which received finam beforehand these phenomena were noticeably weaker in comparison with the mice which did not receive the protector.

The data obtained support the results of the first group of experiments, which showed that the administration of the radio-protector prior to centrifuging makes the dystrophic process caused by the action of transverse acceleration more severe. In addition, it is obvious that the influence of the gravitational factor, before or immediately after irradiation, reduces the severity of radiation damage to the brain and myocardium.

The decrease in the sensitivity to ionizing radiation following a gravitational overload has been described frequently in the literature [6, 9, 26, 27]. The modifying effect was evaluated primarily on the basis of mortality and the average survival time. The morphological studies of this phenomenon are few, and involve primarily the organs of hemopoiesis [1, 2]. The mechanism of the modifying effect is very complicated. It is still far from clear, and has to do with at least 3 factors: hypoxia, accumulation of endogenic sulfhydryl groups, and a change in the functional activity of the regulating systems.

Conclusions

1. The prophylactic administration of the radioprotector finam noticeably reduces the mortality of animals that have been irradiated with sublethal and lethal doses and reduces the degree of postradiation changes in the internal organs.

2. With correct dosage, the preparation produces very slight disturbances of the hyperemia of the organs and rapidly developing changes in the activity of the enzymatic systems, accompanied by moderate swelling of a portion of the mitochondria of the myocardium. There is a decrease in the postradiation physicochemical changes in the protein systems of the myocardium, which is clearly documented by photometry of the preparations that have been analyzed to determine reactive amine groups.

3. Finam not only reduces the degree of postradiation destructive processes, the suppression of nerve regeneration by radiation and the breakdown of the genetic apparatus caused by the development of giant nuclei, but also promotes a more rapid and more complete recovery of the damaged tissues.

4. The administration of a radioprotector prior to the action of transverse acceleration increases the destructive and dystrophic processes, which is most clearly evidenced in the myocardium on the second day of the experiment. /33

5. Preliminary transverse acceleration before gamma irradiation modified the postradiation effect, which consists in a reduction of the dystrophic process in the brain and heart muscle.

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Influence of Pharmacochemical Substances on the Reactivity of the Organism to the Combined Action of Gamma Radiation and Transverse Overloads

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ABSTRACT. Under the combined exposure of mice to gamma radiation from Co^{60} in doses of 350 and 700 r and transverse overloads (10 units, 30 minutes), the authors studied the effectiveness of cystamine and AET in amounts of 75 mg/kg each. The effect of overloads 24 hours prior to irradiation reduces the radiation damage of hemopoietic organs. In mice that were subjected to the action of overloads 24 hours after irradiation, there was an insignificant increase in the radiation damage to the spleen and the marrow and an increase in the destructive changes in the peripheral nervous system. Cystamine combined with AET has an antiradiation protective effect, both for irradiation without overloads and for combined action of radiation and overloads. The combined action of radiation and overloads is characterized by nonuniform damage to various parts of the peripheral nervous system.

An important role in radiobiological studies is played by the search for substances and methods of increasing the reactivity of the organism during radiation. One promising method is the use of pharmacochemical substances and their combinations. In this connection, it is interesting to study the reactivity of the organism under the influence of /336

pharmacochemical substances with the combined action of radiation and overloads.

The purpose of the present paper was to study the pathomorphological changes in the hemopoietic organs and in certain parts of the peripheral nervous system under the combined influence of radiation and transversely directed overloads while using a combination of radioprotective substances.

In the investigations, we used 275 male mice of the S-57 bl strain, weighing approximately 20 grams. Irradiation was performed on the "Khizotron" device with gamma rays from Co^{60} at a dose rate of 18 r/minute. The radiation dose amounted to 350 or 700 r. The transverse overloads with a magnitude of 10 units lasting 30 minutes were imposed singly on a centrifuge with a 4.2 meter arm. Under the combined influence of gamma radiation with overloads, the interval between the action of these two factors amounted to 24 hours in all series.

From the pharmacochemical substances, we selected a combination of cystamine and S, β-aminoethylisothiuronium at the rate of 75 mg/kg each, given 15 - 30 minutes prior to irradiation.

The mice to be examined were killed with ether vapor 6 hours, 1, 3, 7, 15, 30 and 60 days following irradiation. External symptoms of radiation damage (sluggishness, "slovenliness", diarrhea, decrease in eating) disappeared at the beginning of the second week, and somewhat earlier in the case of irradiation at doses of 350 r. During the next 4 - 8 weeks, we observed the formation of patches of grey hair and there were foci of epilation. Among the animals that were selected for

determining mortality, by the 30th day with irradiation at 350 r 10% were dead and at a dose of 700 r — 40%. The death of the mice was observed at periods from 3 - 24 days. Under the combined action of radiation at a dose of 350 r, overloads and protectors, we observed no deaths among the mice. Among the animals which died there were damaged edematous intestines, as well as hemorrhages in the subcutaneous cellular tissue and meninges. Following the combined action of irradiation and overloads, we also frequently found hemorrhages in the heart muscle of the mice. For the investigation, we used a number of neurohistological and ordinary histological methods.

In the animals which were killed for macroscopic investigation, we found (up to the 7th day) damage to the lumen of the intestine and edema of its walls, nonuniform hyperemia of the lung tissue, shrinkage of the spleen and the thymus. On the 7th and 15th days, there were hemorrhages in the lungs and more rarely in the subcutaneous cell structure and intestine; on the 30th day, the spleen in some of the mice was enlarged and tuberous, with foci of a dark-cherry color. /337

In the histological examination of the mice that were subjected to the action of overloads, 6 and 24 hours later we observed venous and capillary plethora of the brain, heart, liver, testes and kidneys. In one animal which was killed after 6 hours, the myocardium was found to contain a small hemorrhagic focus. In the lungs of almost all the mice after 6 and 24 hours, we found plasmorrhexis and escape of erythrocytes into the lumina of the alveoli. In the spleen, we found moderate plethora and suppression of mitotic activity of the hemopoietic cells in the follicles and foci of myelopoiesis. The mitosis patterns were again evident after 24 hours following the action of overloads, and their

subsequent content (on the 3rd and 7th days) increased relative to normal, especially at the foci of myelopoiesis of the pulp, where there was also an increase in the quantity of pyroninophylic reticular cells and hemocytoblasts. After 15 days, we observed a definite increase in the volume of the myelopoiesis foci. In the liver, after 6 hours, we saw a definite decrease in the glycogen content, and drops of fat appeared in the cytoplasm of the cells. After 24 hours, the glycogen content had decreased. Fatty dystrophy of the liver cells remained in some animals up to the 3rd day.

In those animals which received the protectors, after six hours we observed an expansion of the venous sinuses of the marrow, plethora of the spleen and liver, slight peribronchial and perivascular edema in the lungs, and death of a small portion of the cells in the hemopoietic organs. During the next 3 days, we found in the spleen a decrease in the volume of the follicles and foci of myelopoiesis. In the thymus, up to the 7th day there was impoverishment of the cortical substance with respect to thymocytes. In the marrow, up to the 15th day there were fewer hemopoietic cells than usual, and the content of granulocytes with segmented nuclei increased relatively. Until the 7th day, there was a decrease in the pyroninophilia of the germinal cells of the testes and the hepatic cells. In the liver, fatty dystrophy developed, which had disappeared by the 3rd day. In the nerve apparatus of the urinary bladder, we found transient reactive changes in the pulpy nerve fibers; the receptor terminals and their endings remained intact at the same time. Certain nerve conductors were swollen, and we could see fine droplets of sharply impregnated axoplasm in them. These changes in the nervous system were nearly nonexistent after the 7th day.

In those mice which were subjected to gamma radiation at doses of 350 and 700 r, changes in the marrow led to progressive damage to the stroma, which was more severe and developed earlier in the case of irradiation at larger doses. Among the /338 hemopoietic cells, the erythroblasts disappeared earlier than the others, then the myeloblasts, and the juvenile granulocytes. Finally the cells with segmented nuclei disappeared, among which there were many hypersegmented forms. Recovery of the marrow structure began with accumulation of hemocytoblasts around the bone elements and around the endosteum. Then foci or entire dense fields of genetically uniform hemopoietic cells developed - erythroblasts or myeloblasts alone. Sometimes we also found similar foci of megakaryocytes. The tendency to form a focus in terms of hemopoietic cell distribution was evaluated as a stage in incomplete regeneration. Usually by the 15^{th} or 30^{th} days, this focusing phenomenon disappeared and the hemopoietic cells of different types were distributed uniformly through the stroma, which was an indicator of the degree of maturity of the recovery processes. At the same time, however, we did observe recovery of the total number of hemopoietic cells, but young forms predominated among them. Mature cells of the granulocytheric type appeared last of all. In those mice which were subjected to irradiation at a dose of 350 r following the action of overloads, the changes in the marrow were approximately the same as with irradiation at the same dose without the action of overloads. Differences showed up on the 15^{th} day when those mice which were subjected to the combined action, in comparison to those that were merely irradiated, lacked this focusing tendency, and the distribution of the cells in the stroma was uniform. This indicated an earlier and complete regeneration of hemopoiesis in the marrow of these mice.

In those mice that were irradiated with a dose of 350 r with preliminary administration of protectors, the pathological changes in the marrow decreased noticeably and were limited only to impoverishment of the stroma with respect to hemopoietic elements. After 7 days, the total content of cells in the stroma of the marrow had almost completely been restored, and all forms of hemopoietic cells were evident. After 15 days there was a noticeable increase in the content of juvenile forms - erythroblasts and myeloblasts. There were also mature cells of the granulocytheric type, but their numbers were considerably reduced. The usual ratio of hemopoietic cells in the marrow was restored by the 30^{th} day.

When the mice were irradiated at a dose of 700 r 24 hours after the action of overloads, the destruction of hemopoietic cells and the breakdown of the marrow were of the same severity as for only one irradiation at the same dose. At the beginning of recovery (7 days) under combined exposure, the total number of cells was slightly higher. The predominant form of cell elements retained was the erythroblasts, while with irradiation alone there were more myeloblasts. Fifteen days later, we were unable to find a noticeable difference in the structure of the marrow of these two series, but we did notice that with combined action the megakaryocytes were found more frequently and the epiphyseal portions of the femurs were partly filled with cells. After 30 days, these animals lacked signs of /339 focusing, and there were rare occasions of segmented-nuclear granulocytes.

In those mice which received protectors prior to irradiation at a dose of 700 r, the changes in the marrow were of the same nature as for irradiation without prophylactic administration of protectors. However, the severity of these structural

disturbances was considerably less. Twenty-four hours later, there was no destruction, but only impoverishment of the marrow with regard to cellular elements. There were no hemorrhages and no flaking of the endosteum; megakaryocytes were found more frequently, and we could also see mitotically dividing cells. Destruction of the marrow took place only by the 3rd day, but it was evidenced to a lesser degree than for irradiation without administration of protectors. On the 7th day, the marrow was also less damaged and contained more erythroblasts. By the 15th day, the total content of cell elements in the marrow had completely recovered. The hemopoietic cells were represented by erythroblasts, myeloblasts and megakaryocytes, and we did not observe any "focusing" in the distribution of the various forms of cells. It was also possible to find juvenile forms of granulocytes; there were both stabform and segmented nuclei, but more rarely than in the nonirradiated mice. The usual structure of the marrow, with normal ratios of all cell elements, was restored after 30 days.

The changes in the spleen following irradiation at doses of 350 and 700 r led to the breakdown of hemopoietic cells and severe damage to the follicles and pulp with disappearance of myelopoiesis foci from the latter. Recovery of hemopoiesis in the spleen began on the 3rd through 7th days depending on the radiation dose. The mitotically dividing cells, absent after 6 hours, could again be found after several days, but complete recovery of mitotic activity in the elements of the spleen was found 7 days after irradiation at doses of 350 r, and in 15 days after irradiation at a dose of 700 r. In the recovering follicles (in the periphery), we observed reticular cells with pyroninophilic cytoplasm, then lymphoblasts and prolymphocytes; in the last stage the lymphocytes tended to cluster together. The sizes of the follicles gradually returned

to normal. However, their outlines remained unclear for some time, and we could see accumulations of lymphoblasts along the periphery of the follicles. As the lymphocytes accumulated and the volume was completely restored, the limits of the follicles became clear and the accumulations of lymphoblasts "sank" into the depths of the follicles, forming multiplication centers. The recovery of the myelopoiesis foci began with the development of reticular cells with a basophilic cytoplasm in which granules of RNA were revealed by staining with methyl green pyronine. Such cells first appeared on the 3rd through 7th days beneath the capsula and around the trabecula. In the foci, which consisted of pyroninophilic reticular cells, there were hemocytoblasts, and later erythro- and myeloblasts. /340
These foci assume the form of dense fields, as they enlarge, filling a larger portion of the pulp and frequently appearing to suppress the still unformed and shrunken follicles. Judging by the accumulation in these cell fields of the more mature elements, the volume of the myelopoiesis foci decreased to the normal level. These foci were eventually converted into cellular rods of the ordinary type.

Hence, not only the decrease in the size of the follicles but also the lack of clarity in their outlines, the absence of multiplication centers or their location along the edges of the follicles were viewed by us as signs of immaturity of the follicles and suppression of lymphopoiesis in them. The overwhelming content of hemocytoblasts in the foci of myelopoiesis, their location in the pulp in the form of cellular fields or large dense foci were interpreted by us as signs of immaturity of the myeloid hemopoiesis and incomplete recovery. Recovery of the myeloid foci was considered to be complete if they had the shape of cellular rods of moderate volume containing comparatively few hemocytoblasts, many erythro- and myeloblasts

and mature forms of the granulocytheric type.

In those animals which were subjected to irradiation at a dose of 350 r following preliminary exposure to overloads, damage to the spleen was less evident, and recovery of the normal structure was accelerated. After 6 and 24 hours, /342 we noticed a slight indistinct increase in the damage to the follicles and a more severe suppression of the mitotic activity. However, only 3 days later, the regeneration of the myeloid hemopoieses (and after 7 days, the lymphoid type) was more clearly evidenced than for irradiation alone. After 3 days, several of these animals developed small concentrations of erythroblasts in the pulp beneath the capsula; the accumulations of pyroninophilic reticular cells and hemocytoblasts were larger. The most pronounced difference developed after 7 days, while those mice which were subjected to irradiation alone during this period of time developed only small accumulations of hemocytoblasts in the pulp. Those that were subjected to preliminary overloads developed large myeloid foci (Figure 1, 2) which contained not only hemocytoblasts but also myeloblasts, erythroblasts and megakaryocytes. The follicles were also larger in the case of combined action. On the 15^{th} day, the follicles had larger dimensions than those with irradiation alone, and the multiplication centers were also evident. In the myelopoiesis foci, as a rule, we found cells at all levels of maturity, including granulocytes with segmented nuclei. The myeloid cells were distributed in the pulp in the form of rods. The structure of the spleen recovered 30 days following combined exposure.

The administration of protectors to mice before irradiation at a dose of 350 r sharply decreased the radiation damage to the spleen. These differences in the damage to the spleen were /342

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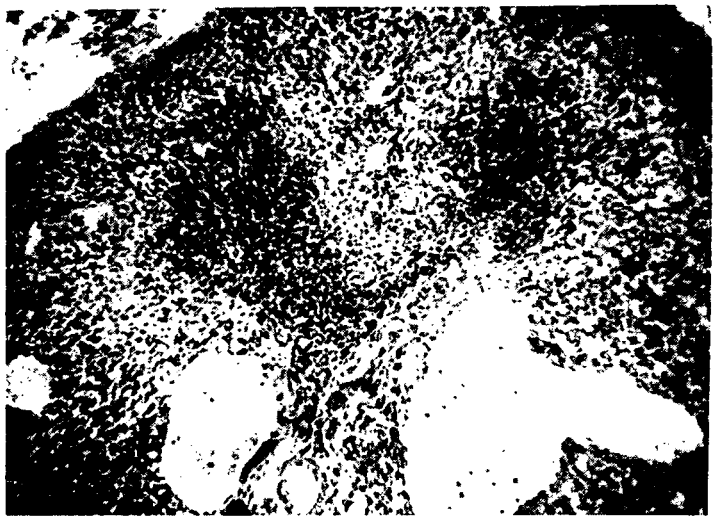

Figure 1. Spleen, 7 days after irradiation at a dose of 350 r. Shrunken follicles are visible, as well as damaged pulp. Hematoxylin-eosin, 105 x, ob. 16.

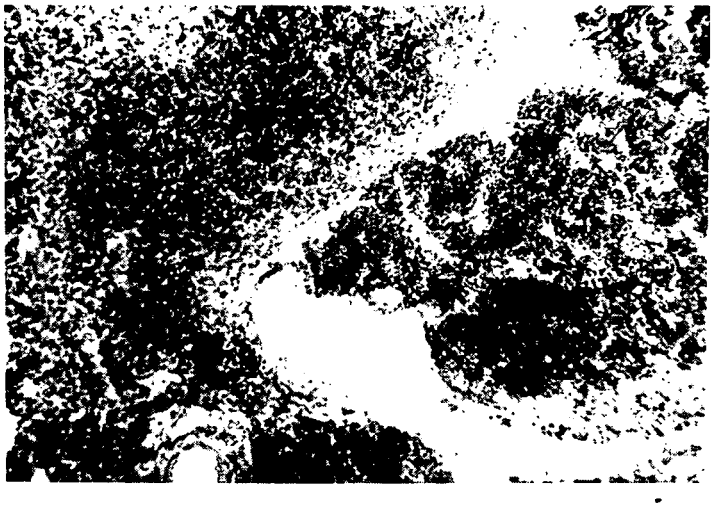

Figure 2. Spleen, 7 days after combined exposure to overload and irradiation at a dose of 350 r. The pulp contains large foci of myeloid hemorrhage. Follicles larger than for irradiation alone (cf. Figure 1). Hematoxylin-eosin, 95 x, ob. 16.

already evident during the first hours following irradiation. After 6 hours, the irradiated mice which had received the protectors had cell fragments essentially only within the centers of multiplication; those that were irradiated without preliminary administration of protectors also developed insignificant amounts of cell fragments in other parts of the follicles. After several days, those mice which had been irradiated following administration of protectors showed less damage to the follicles, while the pulp contained individual erythroblasts. After 3 days the follicles were larger in volume, and some of them contained multiplication centers. In some cellular

accumulations of the pulp, in addition to the hemocytoblasts, we found small accumulations of erythroblasts. Particularly clear differences were found after 7 days. In the pulp of those animals that received protectors prior to irradiation, we found large foci of myelopoiesis, containing not only hemocytoblasts but also myeloblasts and erythroblasts. The follicles were larger. After 15 days, the spleen resumed its normal structure. The follicles had clear limits and formed centers of multiplication. The myeloid cells, in the form of cell rods of moderate size and quantity, were located primarily beneath the capsula and near the trabecula. In the spleen of mice that were subjected to combined exposure to irradiation at a dose of 700 r and preliminary and subsequent overloads, the radiation damage to the spleen was the same as for irradiation at a dose of 700 r.

A significant reduction in the damage to hemopoiesis in the spleen was observed in those mice that were irradiated at a dose of 700 r following administration of protectors. The damage to the follicles was less. After 1 and 3 days the follicles contained lymphocytes in which some of the follicles showed multiplication centers and mitotically divided cells. The pulp contained individual erythroblasts. After 7 days, the follicles (in comparison to the preceding period) increased in size, and lymphoblasts accumulated in them. In the pulp of the spleen, we could see large foci of pyroninophilic reticular cells and hemocytoblasts. By the 15^{th} day, the follicles had regained their shape, and some of them had quite clear limits. The mitosis patterns in some follicles were scattered everywhere, while in others they were localized within the multiplication centers that were formed by this time. The principal mass of cellular elements in the follicles consisted of lymphoblasts and prolymphocytes. In the pulp of the spleen, we could see many cellular bands consisting of hemocytoblasts, erythroblasts

and myeloblasts. By the 30th day, the structure of the spleen resembled the norm. However, the myelopoiesis foci in the pulp remained enlarged, more hemocytoblasts were contained in it than normal, several follicles had unclear limits, and mitosis patterns were found in them beyond the centers of multiplication.

In those mice that were subjected to preliminary exposure /343
to overloads and received protectors prior to irradiation at a dose of 700 r, the dynamics of the morphological changes in the spleen and their severity were the same as for irradiation following administration of protectors, but without the additional action of preliminary overloads.

Significant changes following irradiation at doses of 350 and 700 r were observed in mice in elements of the peripheral nervous system. Following irradiation at a dose of 350 r (after 6 hours), the walls of the urinary bladder showed irritation of pulpy mainly thick (and after several days, also thin) nerve fibers: increased argentophilia, irregularity and serration of the edges, varicosity along the axial cylinders. The pulpless nerve fibers were impregnated more intensively than was normal. On the other hand, impregnation of the gangliar cells was reduced. A similar type of cell-shadow as well as multinucleolate neurons and cells with moderate chromatolysis were largely found in the solar, hypogastric and pelvic nerve plexuses and in the marginal sympathetic chain. By the third day, the destructive changes encompassed a large number of the nerve fibers and neurons of the bladder. In individual pulpy nerve fibers we noticed fragmentation of the axial cylinders. /344
After 7 and 15 days, the morphological changes in the nerve elements of these regions remained qualitatively the same as in the preceding periods, but the severity of these changes had increased. There was an increase in the damage to the

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pericellular apparatus, whose terminal portions grew thicker, and there was homogenization of the synapses. After 30 days following irradiation, there were fewer degenerative changes in the nerve elements in the intramural apparatus. At the same time, we could see changes of a compensatory-accommodative nature: excessive growth of the pericellular junctions with excessive terminal branchings, local growth of nerve fibers of the adventitial and intermuscular nerve junctions (Figure 3), and an increase in the size of the terminal portions of the synapses. Several of the synaptic endings, due to their large size, were in contact with several gangliar cells arranged in rows.

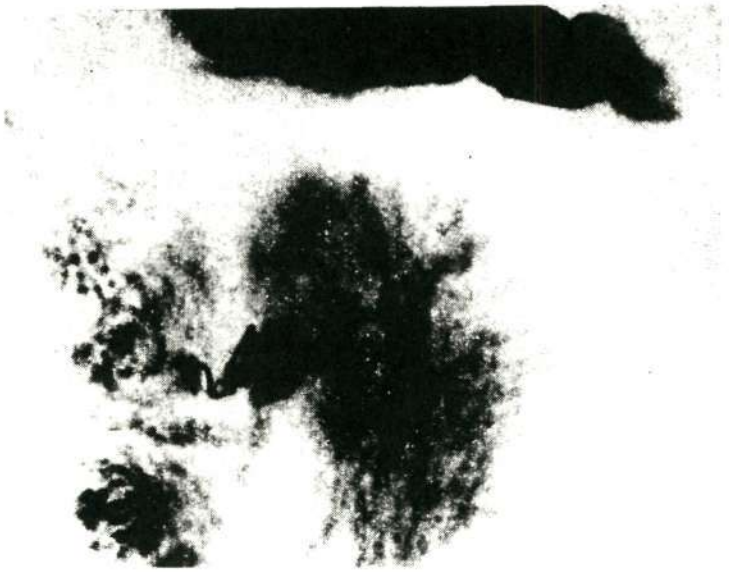

Figure 3. Bladder, 30 days after irradiation at a dose of 350 r. Reactive irritation of nerve fibers in adventitia. Kampos. ob. 10, oc. 12.5.

With an increase in the radiation dose from 350 to 700 r, the pathological changes in the nerve elements were evidenced much more clearly and developed at earlier stages.

If the radiation at doses of 350 or 700 r was preceded by the action of overloads, the damage to the nervous system of the bladder increased in comparison only with irradiation in corresponding doses and also assumed the nature of a mosaic distribution of damaged and unchanged nerve elements. After 24 hours following irradiation with preliminary exposure to overloads, both the

adventitia and the muscular envelope showed excessive destruction of receptor fields (Figure 4). We also found many receptors with different degrees of structural complexity which had both damaged and intact afferent fibers, irritated terminals and terminal portions. At subsequent stages, the damage extended to many neurons of the spinal cord, solar, hypogastric and pelvic nerve plexuses and the intramural junctions of the bladder. However, these changes were not the same in various parts of the nervous system. The anterior horns of the spinal column showed a predominance of hyperchromic and wrinkled nerve cells, while in the posterior portions there were weakly thionine-stained cell-shadows with peripheral and central chromatolysis and pericellular edema. The cells of the spinal ganglia were intensively stained with thionine and contained a fine chromatophilic substance in the neuroplasm as well as many nucleoli. In the solar and pelvic plexuses, in addition to the pulverization of tigroid, some of the neurons showed enlargement of individual grains of chromatophilic substances and the formation of large lumps of Nissl substance. Intramural gangliar cells of the bladder were

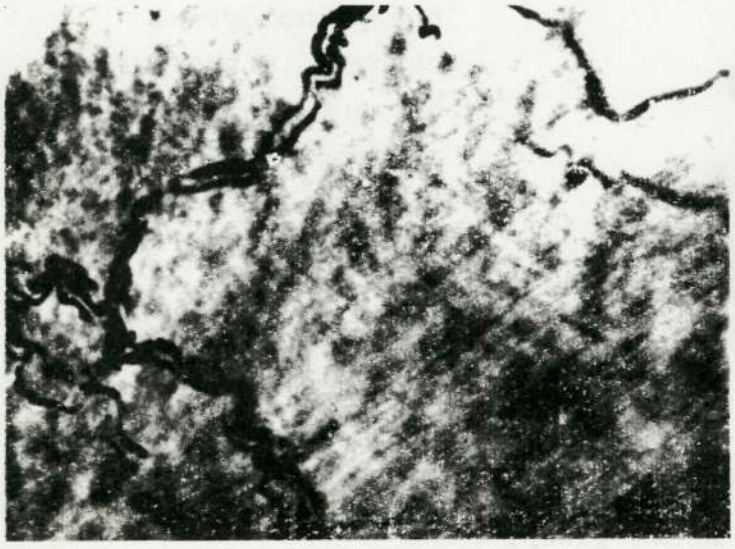

Figure 4. Bladder, 1 day after combined action of overloads and irradiation at a dose of 350 r. Breakdown of afferent nerve junction in adventitia. Kampos. ob. 10, oc. 12.5.

elongated and deformed, and the chromatophilic substance in them was distributed homogenously.

When the animals were irradiated at a dose of 700 r with subsequent overloads, the damage to the nervous system (in comparison to pure irradiation at a dose of 700 r) was intensified. In addition, there were certain qualitative characteristics which led to an increase in the satellitic reaction. /345

The preliminary administration of protectors to mice that were irradiated at a dose of 350 r sharply decreased the severity of the pathological disruptions in all branches of the peripheral nervous system of the pelvic organs. Changes of a reactive nature generally predominated; destructive changes were insignificant in scope both in the nerve system of the bladder, and in the extramural plexuses of the vegetative nervous system, where they occupied only individual thick pulp fibers. These fibers apparently must belong to the spinal cord, inasmuch as they always had connections to the complex receptor endings which are morphologically identical to the spinal ones. Such receptors were found in the sympathetic trunk, solar, hypogastric and pelvic plexuses, in the adrenals, kidneys, mucous membrane of the bladder, and in insignificant amounts in the smooth muscle of the bladder.

Under the influence of protectors, damage to the afferent endings and nerve pathways in the walls of the blood vessels was prevented. In the overwhelming majority of cases, the pathological process in the nerve cells, their synapses, the pericapsular endings, receptors and nerve fibers had recovered by the beginning of the second week. On the 15th and 30th days, the intramural and extramural vegetative plexuses showed a remarkable suppression of pathologically changed neurons, all manner of /346

receptors or intraneuronal connections. Thus, out of 756 neurons that were examined in the solar and pelvic plexuses, two were found to be pyknotic, four with peripheral chromatolysis, six with pyknotic nuclei, i.e., a total of 2.4 ± 0.55%. In the intact animals, out of 563 nerve cells, 8 were found to be pathologically changed, i.e., 1.5 ± 0.51%. The difference was statistically unreliable.

Administration of protectors to mice prior to irradiation at a dose of 700 r also reduced the severity and damage to the intramural and vegetative nervous systems to a lesser degree than with irradiation at a dose of 350 r. However, the nature of these changes at all times was the same as for irradiation at doses of 700 or 350 r, performed without protectors.

When the animals that received protectors prior to irradiation at a dose of 700 r were subjected to the preliminary action of overloads with a decrease in radiation damage caused by the pharmacochemical preparations, we observed a mosaic distribution of altered nerve elements in various parts of it. This mottling in the distribution of the damaged and undamaged portions of the nervous system was described earlier, under the influence of preliminary overloads, without administration of protectors to the irradiated animals. Naturally, in that case the severity of the pathological process was much higher. In those animals that were subjected to the action of a complex of factors (overloads plus protectors plus irradiation at a dose of 700 r), the pathological changes appeared only after 7 days.

Hence, in those mice that were subjected to gamma irradiation at a dose of 350 r, preliminary action of overloads reduced the damage to hemopoiesis in the marrow and especially the spleen. Recovery of hemopoiesis was accelerated: by the seventh day

following this type of combined action, the pulp of the spleen showed large foci of myelopoiesis; the follicles were larger than in the case of irradiation alone. The positive influence of overloads on the reaction of hemopoiesis in the irradiated organism may be due to a number of causes. Overloads produce severe hypoxia of the mixed type in the organism, caused by the redistribution of blood in the organs and the disruption of oxygenation in the lungs [3, 6, 9, 17 - 19, 29]. The oxygen consumption drops [12]. A single exposure to overloads at a value of 25 units causes a drop in the oxygen consumption in the liver and diaphragm by 84 - 86% [11].

In the course of 24 hours following the action of /347
overloads, the brain, myocardium, liver, testes, kidneys and hemopoietic organs of the mice showed stagnant plethora, which could maintain and retain all those changes that arose during acute hypoxia during the action of overloads. Therefore, there is a basis for assuming that the influence of overloads on the radiation damage to the hemopoietic organs may be due to the oxygen effect. A drop in the consumption of oxygen at the time of irradiation, as we know, weakens the primary radiobiological effect [8, 13, 14, 16, 21]. Data are available indicating that, following the hypoxia, the mice showed phasal changes in the radiosensitivity: an increase in the period from 3 to 18 hours following the end of hypoxia and a decrease after 24 - 72 hours [23]. Fatty dystrophy of the liver and a decrease of the glycogen content in it indirectly support the presence of hypoxic conditions. At the same time, accumulation of fatty substances, as shown by the study of V. M. Alekseyeva [1], may reduce the radiosensitivity of the organism due to the ability of fats to oxidize readily and thereby prevent the access of oxygen to more important cell structures.

The radiosensitivity of the cells is increased with an intensification of their proliferative activity [15]. Therefore, one of the mechanisms of reducing the radiation damage under the influence of preliminary overloads is the decrease in the mitotic activity of the hemopoietic cells in the spleen and marrow which they may cause.

Preliminary action of overloads during irradiation at a dose of 700 r leads to much less noticeable changes in the damage to hemopoietic organs. A slight decrease in the damage to the structures of the spleen and marrow, which was noticed in mice following the action of overloads, obviously is of no importance for the outcome of radiation sickness at this radiation dose.

This is especially true of the action of overloads after several days following irradiation at a dose of 700 r. Under a combined effect of this nature, the damage to the marrow and spleen is not reduced, but even increased slightly due primarily to the more pronounced initial postradiation damage to these organs. Obviously, the overloads that are administered several days after irradiation, increasing the load on the cardiovascular system, lead to mobilization of the hemopoietic cells that are retained at this time and their evacuation in the vascular tree.

The results of studying the animals that died shows that the overloads, both preliminary and subsequent, combined with irradiation at a dose of 700 r, increased the hemorrhagic phenomena of radiation sickness. Hemorrhages in these animals developed not only in the lungs and the subcutaneous cell structure, but also in the myocardium. These data agree with studies in which it was shown that irradiation of the animals under various stress or fatigue conditions leads to more severe

damage of the myocardium and to more frequent development of hemorrhages in the heart muscle [4, 5].

A characteristic feature of the structural changes in the peripheral nervous system under the combined influence of radiation and overloads is the nonuniformity of its damage in various organs and within the limits of a single organ.

As we mentioned earlier, under the influence of overloads the blood moves in the direction of the vector of centrifugal forces, so that in some organs sluggish hyperemia develops, while in others there is extreme anemization. According to the data of Gell and Cranmore [20], under the influence of overloads these differences in hyperemia may arise in the vessels of a single organ. It is probable that for this reason the reactivity of various parts of the nervous system under different hemodynamic conditions also changes differently, as evidenced at the time of irradiation by a nonuniform radioresistance of the nerve elements of a given type. The mosaic nature of the damage to the peripheral nervous system was noted with the combined action of overloads with irradiation at doses of 350 and 700 r. The action of subsequent overloads, retaining the same mosaic type of damage as under the influence of preliminary overloads, increases the destructive changes in the elements of the peripheral nervous system.

Cystamine and S-,β-aminoethylisothiuronium, when administered in a single intraperitoneal dose to mice, produce circulatory changes in many organs, death of hemopoietic cells and suppression of the mitotic activity in the marrow and spleen, as well as reactive changes in the elements of the peripheral nervous system. A number of changes developed that indicate changes in metabolism (fatty dystrophy of the liver, decrease in the RNA

concentration in the cells of the liver and testes).

Administration of these preparations prior to irradiation sharply reduces the severity of the pathomorphological changes in the hemopoietic organs and in various portions of the intramural and vegetative nervous systems of the peritoneal cavity and pelvis. Recovery of the normal structure of the hemopoietic organs was complete by the 15^{th} day with irradiation with 350 r, and by the 60^{th} day with irradiation at a dose of 700 r. The damage to the nervous system was limited to the development of reactive changes, but the destructive changes were insignificant for only 1 week.

The preliminary action of overloads did not reduce the action of the protectors. Under the complex influence of preliminary /349
overloads, protectors and gamma irradiation at a dose of 700 r, the radiation damage to the hemopoietic organs and the peripheral nervous system was reduced just as in the case of irradiation with protectors, but without preliminary centrifuging. In this case, the nonuniform nature of the changes in the nervous system organs, which was noticed under the influence of overloads and irradiation without protectors, also appeared with the administration of protectors.

Consequently, a physical factor such as overloads significantly changes the radioresistance of the organism regardless of whether or not it acts prior to irradiation at a dose of 350 or 700 r, or even at a dose of 700 r with preliminary administration of cystamine and AET in equal doses.

Conclusions

1. The action of overloads at a magnitude of 10 units lasting 30 minutes and given 24 hours prior to gamma irradiation at a dose of 350 r reduces the radiation damage to the marrow and especially the spleen, speeding up the structural recovery of these organs. Under the influence of overloads several days prior to irradiation with a dose of 700 r, the effect of reducing the radiation damage is less pronounced.

2. Under the influence of overloads administered several days after irradiation at a dose of 700 r, there is an insignificant increase in the primary radiation damage to the spleen and marrow, and there is an increase in the destructive changes in the peripheral nervous system.

3. Cystamine and AET, with simultaneous intraperitoneal administration in amounts of 75 mg/kg each, 15 to 30 minutes prior to gamma irradiation, at doses of 350 or 700 r, sharply decrease the severity and depth of the pathomorphological changes in the hemopoietic organs and in the nervous system of the pelvis and peritoneal cavity organs.

4. Preliminary overloads administered 24 hours prior to irradiation at a dose of 700 r do not change the protective effect of a mixture of cystamine and AET.

5. A characteristic feature of the action of overloads on the peripheral nervous system of the irradiated organism is inhomogeneous damage to various parts of it.

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N73-20087

The Problem of the Resistance of Experimental Animals To Acute Hypoxia at Various Stages of Radiation Sickness

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ABSTRACT. In experiments on white rats, the authors studied the influence of x-radiation on the resistance of the animals to anoxic, anemic and histotoxic hypoxia. At the same time, they studied the histophysiological state of the adrenal cortex. Increased resistance of the irradiated rats to hypoxia was caused by the nonspecific reaction of the organism to the stress effect, realized through the adrenal cortex.

One of the characteristic features of radiation damage is /350 the disturbance of the accommodative-regulatory mechanisms resulting in a change in the reactivity of the organism. Studies conducted recently indicate the possibility, not only of a decrease, but also of an increase under certain conditions in the resistance of irradiated animals to the action of environmental factors.

The mechanism of this effect has not been studied especially. The opinion has been voiced that the basis for it is the distinctive disruption of protein metabolism [6, 7], action on the central nervous system [5], and a drop in the intensity of metabolism [1].

Without denying the role of the central nervous system and the changes in metabolism in the increased resistance of an organism following irradiation, we have suggested that it is due to the system of the hypophyseal-adrenal cortex system, which plays an important role in the increase of the non-specific resistance [4]. It is now well-known that x-radiation causes nearly the entire symptom complex associated with the adaptation-trophic syndrome.

The purpose of this paper was to study the influence of /351
x-rays over a wide dose range on the resistance to acute hypoxia of various types (anoxic, anemic and histotoxic). At the same time, we studied the histophysiological state of the adrenal cortex.

This study was significant from both the theoretical and practical standpoints. A knowledge of the nature and the mechanisms of the changes in resistance of an irradiated organism is required for solving the problems of the combined action of environmental factors. In addition, this type of investigation has made it possible to understand certain characteristics of radiation stress, among other influences that cause the "stress" reaction.

The experiments were performed on male white rats weighing 120 - 140 grams. The animals were irradiated with the RUM-3 apparatus, 10 animals being exposed simultaneously under the following conditions: voltage of 180 kV, current intensity of 15 μa, filters 0.5 mm Cu and 1 mm Al, skin-focus distance 60 cm, dose rate 39.3 r/min.

To study the effect of hypoxic hypoxia, the animals (experimental and control simultaneously) were placed in a barochamber

and kept there for 10 - 12 minutes at a residual pressure of 100 mm Hg. Hemic hypoxia was produced by placing the animals in a gas chamber containing 8 mg/l of carbon monoxide. Tissue hypoxia was produced by subcutaneous administration of potassium cyanide at the rate of 5 mg/kg. The survival rate of the experimental animals was calculated on the basis of the comparison with the controls. In studying the resistance to hypoxic hypoxia, the rats were irradiated with various doses of x-rays — 50, 100, 200, 500, 700 and 1000 r. The "rise in altitude" was accomplished at various times following irradiation, i.e., after 3 hours, 1, 2, 3, 5, 6, and 10 days. The resistance of the animals to carbon monoxide and potassium cyanide was measured 24 hours after irradiation at a dose of 700 r.

We studied the adrenals of the rats morphologically after the animals had been exposed to various doses of x-rays and at various times after exposure. The adrenals were fixed in a Kaiserling solution, after which sections were prepared from them using a freezing microtome. The sections were then studied either in a stained condition (Sudan black) or unstained in polarized light. This made it possible to evaluate the content and the distribution in the adrenal cortex of sudanophilic and doubly-refracting lipids. The thickness of the adrenal cortex was measured in arbitrary units (based on the weight of the rat). The measurement was performed using a projection of the section through an enlarger at 4 opposite points.

The data on the survival of the irradiated animals "at altitude" and following poisoning with carbon monoxide and potassium cyanide are shown in Table 1. It is clear that, beginning with a dose of 200 and extending up to 700 r (inclusive), the animals were more resistant to acute hypoxia of the anoxic type. Irradiation with smaller (500 and 100 r) and larger /353

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TABLE 1. EFFECT OF X-RADIATION ON SURVIVAL OF WHITE RATS UNDER CONDITIONS OF ACUTE HYPOXIA

Experimental conditions		No. of animals	Survived		Died	
			Abs.	%	Abs.	%
"Rise in altitude" 3 hours after irradiation at dose of 700 r	Experimental	29	17	61.7	12	38.2*
	Control	29	8	27.6	21	72.4
Same, 1 day later	Experimental	42	26	61.9	16	38.1*
	Control	42	13	30.9	29	69.1
Same, 2 days later	Experimental	40	14	35.0	26	65.0
	Control	40	8	20.0	32	80.0
Same 5 days later	Experimental	25	14	56.0	11	44.0
	Control	25	10	40.0	15	60.0
Same, 6 days later	Experimental	25	9	36.0	16	64.0
	Control	25	11	44.0	14	56.0
Same, 10 days later	Experimental	24	4	16.0	20	84.0
	Control	24	8	33.3	16	66.7
"Rise in altitude" 1 day after irradiation at dose of 50 r	Experimental	15	5	33.3	10	66.7
	Control	15	6	40.0	9	60.0
Same, 100 r	Experimental	20	8	40.0	12	60.0
	Control	20	10	50.0	10	50.0
Same, 200 r	Experimental	20	11	55.0	9	45.0
	Control	20	7	35.0	13	65.0
Same, 500 r	Experimental	25	14	56.0	11	54.0
	Control	25	8	32.0	17	68.0
Same, 1000 r	Experimental	15	4	20.6	11	79.4
	Control	15	5	33.3	10	66.7

(Table continued on following page)

TABLE 1 (Continued)

Experimental conditions		No. of animals	Survived		Died	
			Abs.	%	Abs.	%
Poisoning with carbon monoxide 24 hrs after irradiation at dose of 700 r	Experimental	20	15	75.0	5	25.0*
	Control	20	9	45.0	11	55.0
Poisoning with potassium cyanide 24 hrs after irradiation at dose of 700 r	Experimental	14	9	64.3	5	35.7*
	Control	14	4	28.5	10	71.2

* $\overline{p} < 0.01$.

(100 r) doses did not have a protective effect. The maximum protective effect was obtained at a dose of 700 r.

The increase in resistance with irradiation of 700 r was observed even after only 3 hours following the exposure and was retained up to the 5th day (inclusive). On the 6th and 10th days of the protective effect, we did not observe any anoxic hypoxia. Under the influence of radiation at a dose of 700 r, there was a significant increase in the resistance to the toxic effect of carbon monoxide and potassium cyanide.

Without discussing the results of the experiments in terms of survival, let us move on to a discussion of the data that were obtained in studying the histophysiology of the adrenal

cortex of the irradiated animals. We studied the adrenals of 50 rats that made up 10 groups of 5 rats each. The distribution of the animals by groups, depending on the radiation dose and the time which had elapsed since irradiation, as well as the data on the weight coefficients of the adrenals and the thickness of their cortical layer, are all shown in Table 2.

TABLE 2. INFLUENCE OF X-RADIATION ON WEIGHT COEFFICIENT OF ADRENALS AND THEIR CORTICAL THICKNESS IN WHITE RATS*

Irradiation, r	Time killed after irradiation, days	Weight coefficients	Thickness of cortex, arbitrary units	Irradiation, r	Time killed after irradiation, days	Weight coefficients	Thickness of cortex, arbitrary units
Control		0,21±0,1	58±5	700	2	0,39±0,03	91±6
100	1	0,19±0,02	51±5	700	3	0,42±0,03	83±13
1000	1	0,24±0,07	63±12	700	4	0,49±0,05	78±10
700	3 hrs	0,37±0,03	72±8	700	6	0,40±0,06	99±9
700	1	0,31±0,03	89±6	700	10	0,41±0,02	95±6

*Commas represent decimal points.

It is evident from the table that in those rats that were irradiated with x-rays at a dose of 100 and 1000 r and killed 24 hours following irradiation, the weight coefficients and the thickness of the adrenal cortex were practically constant. In those rats that were irradiated with x-rays at a dose of 700 r, 3 hours after exposure we noticed a statistically significant increase in the weight coefficient of the adrenals and the hypertrophy of their cortex, and on the 2nd, 3rd, 4th, 6th and

10th days the weight coefficient of the adrenals exceeded that for the control group of rats by almost a factor of 2.

The histological changes that were observed in the cortex of the adrenals of the rats irradiated with a dose of 100 r and killed after 24 hours were insignificant. They were expressed in a slight decrease in the sudanophilic lipids in the external portions of the fascicular zone (in 2 out of 5 rats) and a slight impoverishment of the latter with respect to doubly-refracting lipids (in all 5 rats).

Similar changes in the content of sudanophilic lipids were observed in those animals that were killed 24 hours after irradiation at a dose of 1000 r. However, in contrast to the preceding group, these animals showed a sharp decrease in the content of doubly-refracting lipids. There was also a change in the aggregate state of the lipoid inclusions in the protoplasm of the cells of the fascicular zone. Instead of fine granules, there were large drops, which occupied almost the entire protoplasm. These changes were observed only in the group of animals that was irradiated at a dose of 1000 r. In those animals that were irradiated at a dose of 700 r and examined after 3 hours, as well as on the first through fourth days, we observed a gradual decrease in the content of sudanophilic and doubly-refracting lipids in the fascicular zone of the cortex, until they disappeared completely on the fourth day. Those rats that were killed on the 6th and especially on the 10th day following irradiation with 700 r, showed a sharp increase both in the sudanophilic as /354
well as the doubly-refracting lipids. In this case, the fatty infiltration occupied all the layers of the cortex including the reticular zone where there are normally few lipids.

The data of the histophysiological studies indicate an increase in the functional activity of the adrenal cortex during the first 4 days following irradiation in a dose of 700 r. In those rats that were irradiated with a dose of 700 r and killed on the 6th and 10th days following irradiation, the adrenals remained sharply hypertrophied, and the thickness of their cortex was twice as great as normal. However, in contrast to those animals that were killed at earlier stages, the adrenal cortex showed a sharp increase in the content of sudanophilic and doubly-refracting lipids, i.e., we observed a picture that was the direct opposite of that which indicated hyperfunction of the adrenal cortex.

We mentioned earlier that on the 5th day the irradiated animals no longer had increased resistance to anoxic hypoxia, which, as we can see from the above, coincides in time with the period of the hyperfunctional state of the cortex. Therefore, we can suggest with considerable certainty that the morphological changes that we observed on the 6th and 10th days following irradiation at a dose of 700 r indicate a sharp drop in the activity of the adrenal cortex. The appearance in the cortex of a large quantity of lipids probably indicates insufficient utilization in the synthesis of hormones.

The increase in the resistance to hypoxia was observed in the dose range from 200 to 700 r. Irradiation at smaller (100 r) and larger (1000 r) doses did not produce this effect. These facts also are explained in the data of the histophysiological investigations. It was found that a dose of 100 r produces only /355
a mild reaction in the cortex, one which is apparently insufficient to develop increasing resistance. A dose of 1000 r causes severe dystrophic changes in the cells of the cortex, but the latter do not hypertrophy. This obviously indicates a sharp

inhibition of its functional activity, which occurs in the absence of the protective effect.

Hence, a comparison of the data on the change in the resistance to hypoxia with the results of histophysiological investigations provides a basis for assuming that increased resistance of irradiated rats to hypoxia is caused by a non-specific reaction and is accomplished through the adrenal cortex.

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N75-20088

State of the Peripheral Blood of Irradiated Animals under Prolonged Conditions of Reduced Barometric Pressure

N. I. Yezepchuk and N. N. Dobrov

ABSTRACT. The authors studied radiation reactions (450 r) of rats kept at reduced barometric pressure (198 mm Hg, altitude 10,000 m, partial pressure of oxygen close to that on the ground). The conditions of reduced barometric pressure had a negative effect on the survival rate of the irradiated rats. The preliminary "acclimatization" of the animals to the altitude slightly modified the effect of radiation sickness.

There were no significant differences in the blood indices in the "acclimatized" and "nonacclimatized" rats that were irradiated.

The study of radiobiological effects that arise under the /355
influence of ionizing radiation on the organism when the latter is under the conditions of an altered gas medium and barometric pressure is of definite interest.

The changes in the composition of peripheral blood constitute one of the indices for evaluating the influence exerted on the organism by ionizing radiation, as well as altered conditions of the gaseous medium or barometric pressure.

Considerable space is devoted in the literature to the general features of changes in the blood system during radiation damage of varying severity. Data are available on the changes in the morphological composition of the blood under the combined /356
action of radiation and reduced barometric pressure. In this case, there is a decrease in the resistance of the organism to changes in atmospheric pressure in irradiated mice. Thus, A. I. Zhuravlev [7], V. A. Volkova and M. T. Turkmenov [5] mention the dependence of the sensitivity of irradiated mice to reduced barometric pressure on the irradiation times or the phases of radiation sickness. Z. I. Barbashova [1, 2] established that preliminary "acclimatization" to altitude enables animals to better withstand the effect of radiation, and explained this phenomenon by an increase in the total resistance of the organism to radiation in animals that have become adapted to altitude. In an earlier work, Z. I. Barbashova [1] mentions the deterioration of radiation sickness under acute oxygen starvation. The author mentions that brief hypoxic conditions produced in the animals before or after irradiation also complicate the course of radiation reactions. Several authors have expressed the viewpoint that preliminary "acclimatization" to altitude has a favorable influence on the course of radiation sickness [8]. Yu. A. Zhukov [6] noticed reduced deaths of white mice that were placed under conditions of reduced barometric pressure after irradiation in comparison to those animals that were kept under ordinary conditions following exposure to radiation. M. M. Mikhaylov [9, 10] observed leucocytosis, monocytosis and neutropenia in rats during the first 10 days of life at altitude, at the same time that irradiated rats that were kept under conditions of reduced barometric pressure showed pronounced leucopenia due to a drop in the lymphocytes during the same time. Among those animals that were placed in an environment with reduced barometric pressure after irradiation, the deaths began earlier and were greater than in

the animals that were merely irradiated or "acclimatized" beforehand to altitude. Radiation damage is mildest in the case of preliminary acclimatization to altitude and subsequent irradiation, as indicated not only by the survival or the animals but the state of the hematological indices. G. A. Vasil'yev [4] mentions the more favorable course of radiation sickness in dogs that have been "acclimatized" to altitude, expressed in particular by a more developed leucopenia, earlier and more rapid recovery of leucocytes, and a larger quantity of thrombocytes on all observation days.

The conditions of reduced barometric pressure without hypoxia, according to the data of Zhuravlev, somewhat ease the radiation damage or even act as a method of protection against it. The materials from the small amount of data contained in the literature indicate that first keeping the animals under reduced barometric pressure does not complicate the course of radiation sickness. These materials fail to give sufficient information about the combined effect on the organism of radiation and reduced barometric pressure with various time-variant combinations of these two factors. /357

In order to study the course of radiation reactions under conditions of prolonged maintenance of the animals at altitude, we performed a series of experiments on male white rats. The altitude was 10,000 m (barometric pressure of 198 mm Hg). The partial pressure of oxygen was close to that on Earth. The animals were irradiated at a dose of 450 r using a cobalt source (Co^{60}) at a dose rate of 28 r/min. The conditions under which the experiments were performed were as follows:

I	5 animals	Irradiation.
II	5 animals	Irradiation with subsequent maintenance for 57 days in a barochamber.
III	6 animals	Preliminary maintenance in a barochamber for 36 days; irradiation with subsequent maintenance under vivarium conditions.
IV	5 animals	Preliminary maintenance in a barochamber for 36 days; irradiation with subsequent maintenance at altitude for 57 days.
V	5 animals	Kept in barochamber for 98 days.

The principal index for evaluating the radiation damage was the state of the peripheral blood of the animals. A hematological investigation was performed (using generally employed methods) twice prior to the beginning of the experiment, on the second, 7^{th}, 14^{th}, 22^{nd}, and 57^{th} days following irradiation, and 2 - 3 days and 10 days after the end of the experiment. In the dynamics we observed the quantity of hemoglobin (in grams %), erythrocytes (in millions), leucocytes (in thousands), the absolute quantity of eosinophils, reticulocytes (in %) and thrombocytes (in thousands).

It should be mentioned that the maximum mortality (60%) of the rats was in group II, where the radiation took place before the rats were placed in a barochamber, and the animals died on the 14^{th} through 15^{th} days following irradiation.

The original values for the indices of the peripheral blood in all of the rats did not exceed the limits of the physiological

norm for this species of animal. During the experiment, there were significant changes in the morphological composition of the blood. In all of the irradiated rats, regardless of the period of time they spent at the altitude, even on the second day following the exposure to radiation we observed a drop in the content of hemoglobin and erythrocytes in comparison with the original values. These changes were particularly noticeable on the 7th through 22nd days following irradiation in the animals of groups I and III (Figures 1, 2). From the 7th through the 14th days, the number of erythrocytes began to rise, and by the 52nd day it reached the original values, except for the rats in group III. The hemoglobin content in the rats of groups II and III began to increase on the 14th through the 22nd days, but did not reach the original values until the end of the experiment. /359

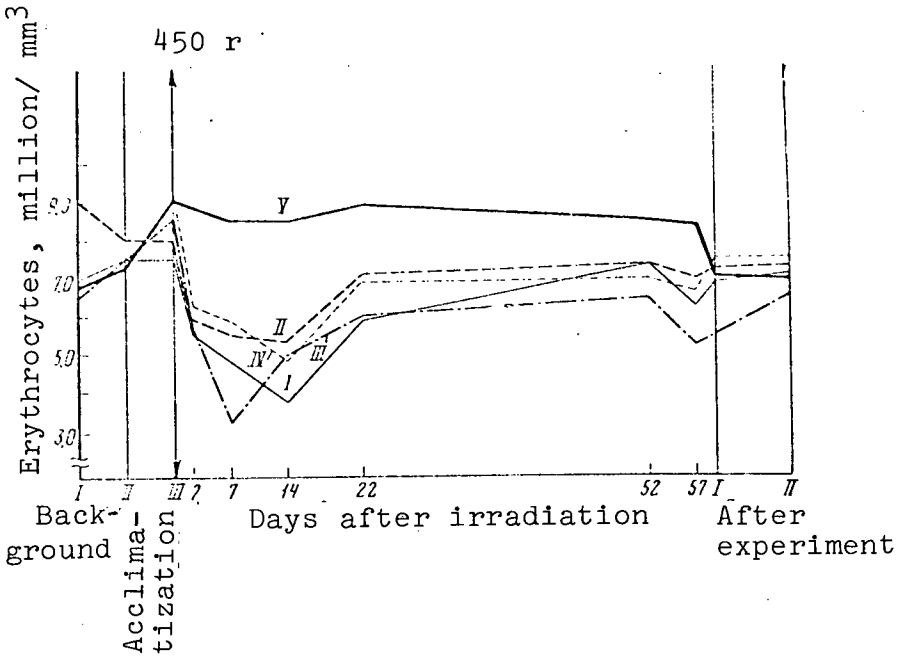

Figure 1. Change in number of erythrocytes under the combined action of irradiation and hypoxia:

I — 450 r; II — 450 r + barochamber; III — barochamber + 450 r; IV — barochamber + 450 r + barochamber; V — barochamber

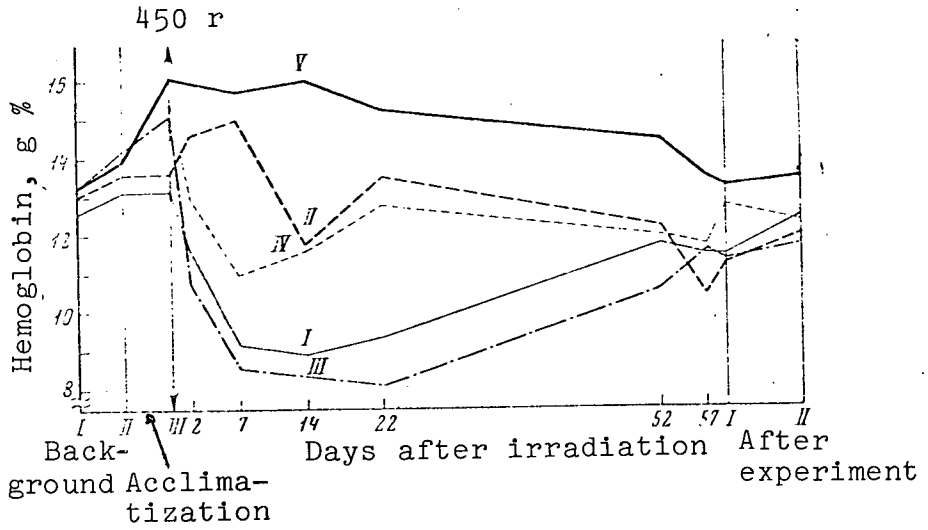

Figure 2. Hemoglobin content under the combined action of irradiation and hypoxia:

Symbols are the same as in Figure 1

The change in the content of reticulocytes in all of the irradiated animals took place most markedly (Figure 3). In the rats of group III, which were kept under conditions of acclimatization to altitude, the values of this index at the time of irradiation were much higher than the original. After 2 days following radiation exposure, the animals of all the groups showed a sharp drop in the number of reticulocytes. During the last 14 to 20 days following irradiation, the rats of all the groups showed an increase in the reticulocyte content. The recovery process of this particular index was most rapid in the rats of groups I, III and IV. In the surviving rats of group I, the remarkable increase in the content of reticulocytes on the 22nd day following irradiation was replaced by normalization of this index 52 days following exposure, after which the number of reticulocytes again became higher than the original level, where it remained even after the end of the experiment. In the rats of group V, the quantity of reticulocytes was uniformly increased during the entire observation period by 56 to 60% in

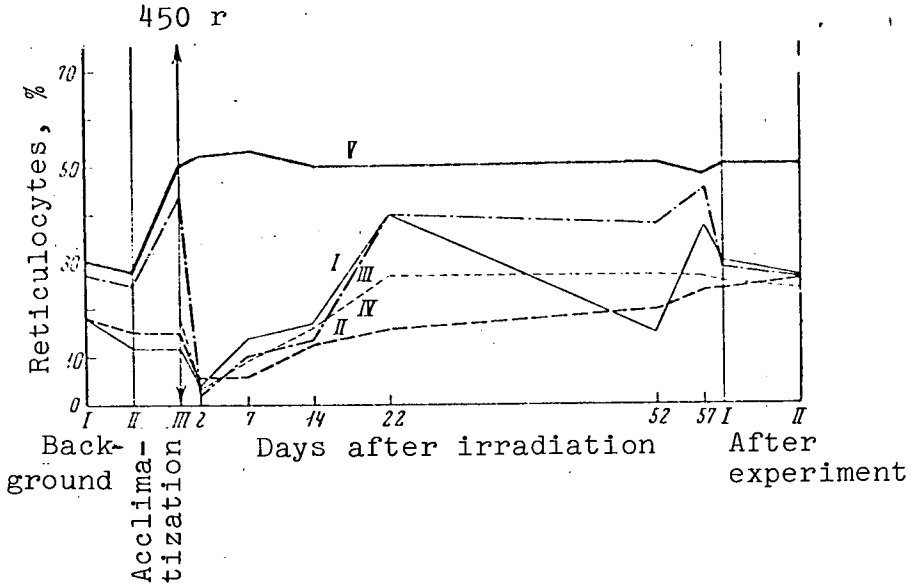

Figure 3. Change in the number of reticulocytes under the combined action of irradiation and hypoxia:
Symbols are the same as in Figure 1

comparison with the original level. The period of recovery in all the experimental animals was accompanied by the appearance in the peripheral blood of juvenile forms of erythrocytes — polychromatophils and normoblasts. As far as the indices in the white blood picture were concerned, we also noticed some significant changes. On the second day following irradiation exposure, the majority of the animals showed a sharp increase in the number of leucocytes (by 90% on the average) and remained at this level for up to 14 days (Figure 4). With a comparative evaluation of the recovery of the number of leucocytes, we can see that the increase in the number of leucocytes in the rats that were irradiated following preliminary stay in a barochamber (group III) took place at a more rapid rate, and the original level was reached by this index already by the 22nd day following the

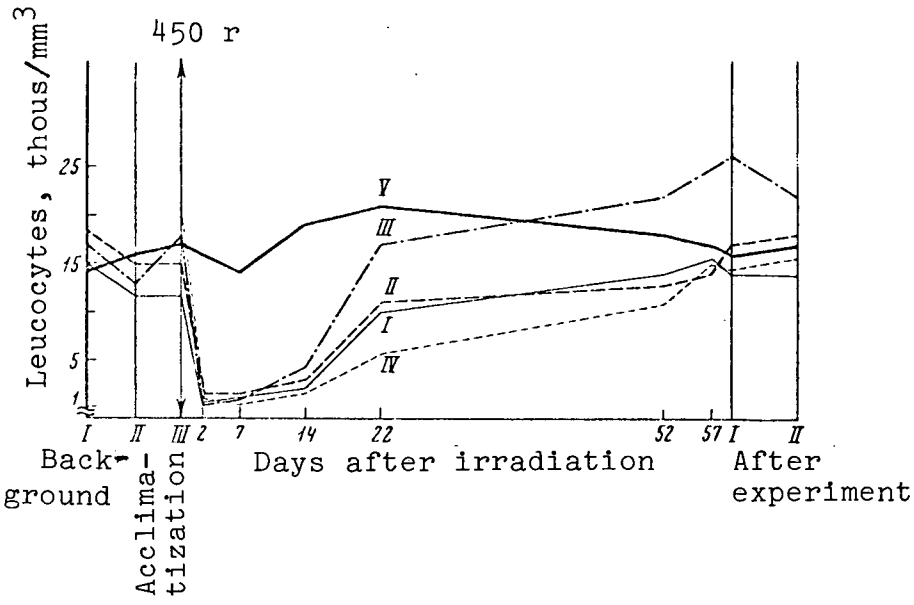

Figure 4. Change in number of leucocytes under the combined action of irradiation and hypoxia:

Symbols are the same as in Figure 1

action of radiation. The slowest and most monotonic recovery of the number of leucocytes was found in the rats of group IV, which were again placed in the barochamber following acclimatization to altitude and irradiation. Important changes in the leucocyte formula, expressed in the sharp decrease in the quantity of lymphocytes due to the relative neutrophilesis, were observed on the second day following irradiation in all of the irradiated animals, while in the "acclimatized" rats the lymphopenia was much less pronounced than in the "non-acclimatized" animals.

The cytological changes in the peripheral blood had a degenerate nature, typical of radiation sickness, and took the form of remarkable cytolysis of the cells, hypersegmentosis of the nuclei of the neutrophils, appearance of giant neutrophils,

pyknosis of the nuclei of the lymphocytes, and karyorrhexis. These changes were manifested mainly on the 7th through 14th days following radiation in all of the experimental rats, regardless of how the animals were maintained later. By the 20th day /361 the degenerative cells had vanished from the peripheral blood.

The absolute quantity of eosinophils in practically all observation periods underwent changes that matched the changes in the quantity of leucocytes. Thus, in all of the irradiated animals on the second day following exposure to radiation, we noticed significant eosinopenia, extending as far as complete disappearance of eosinophils from the peripheral blood of certain animals (Figure 5). This picture was retained for 7 - 14 days, after which the value of this index began to recover slowly in all groups of animals except group I, where the number of eosinophils on the 22nd day was considerably in excess of the original level (by 240%). In the rats of group II, on the 57th day the number of eosinophils recovered most slowly in the "acclimatized" animals which were kept at an altitude after radiation (group IV).

The absolute quantity of thrombocytes in all of the irradiated animals decreased sharply on the 7th through 14th days following the action of radiation, and only after 20 days did it begin to increase slowly, remaining below the original values in almost all cases until the end of the experiment and even after it was over.

An analysis of the material presented will show that irradiation of animals in all experimental groups at a dose of 450 r led to significant changes in the state of the peripheral blood which was expressed in acute leucopenia, eosinopenia, thrombocytopenia, and reduction of the hemoglobin content and number of erythrocytes during the first days following radiation.

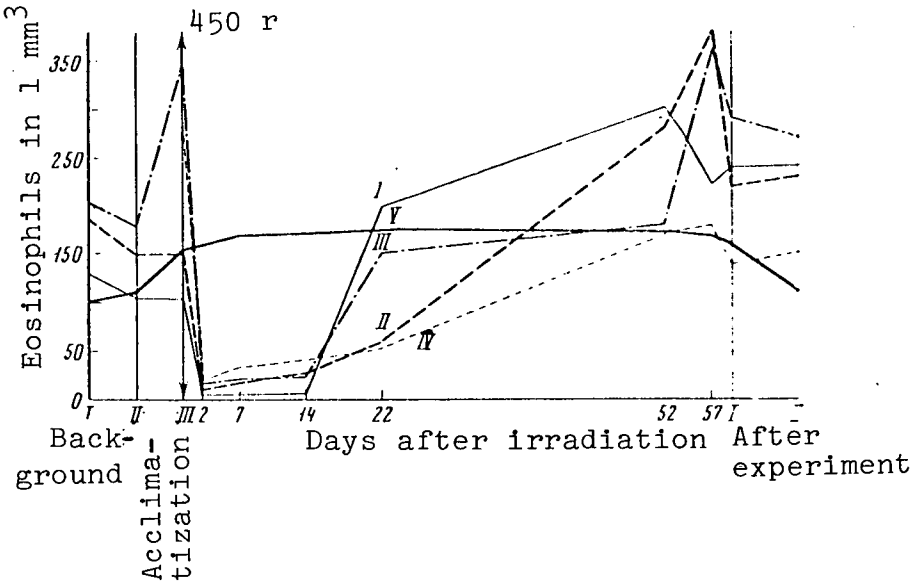

Figure 5. Absolute content of eosinophils in blood under the combined action of irradiation and hypoxia:

Symbols are the same as in Figure 1

In the rats of group IV, in comparison with group III, we observed a less pronounced decrease and a more rapid increase in the quantity of hemoglobin and erythrocytes. Reticulocytosis during the recovery period was less pronounced in the animals of group IV, which also indicates a less severe disruption of the erythropoietic function in the early stages following irradiation in the rats previously "acclimatized" to altitude. At the same time, however, the number of leucocytes and eosinophils was more slowly restored in the animals of group IV. Placing the irradiated animals under reduced barometric pressure had a negative effect on the survival rate of the rats. However, among the animals in group II that survived, we noticed relatively less pronounced changes in the number of erythrocytes and hemoglobin than in the animals that were adapted to altitude. Hence, in contrast to the data of certain authors [1, 2, 8], who indicate

a higher retention of hemoglobin and erythrocytes following irradiation of "acclimatized" animals, we did not succeed in finding important differences in these indices in the "acclimatized" and "non-acclimatized" animals. Here obviously we must keep in mind the specific conditions of our experiment in which the partial pressure of oxygen was close to that on the ground, and a hypoxic condition practically did not develop at all in the animals.

Preliminary "acclimatization" of animals to altitude in our experiments somewhat modified the radiation sickness.

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METHODOLOGICAL ARTICLES /363

Method of Measuring the Composition and Intensity of the Heavy Component of Cosmic Radiation

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S. P. Tret'yakova

ABSTRACT. For expeditious calculation of impacts of heavy nuclei on a target, a method was worked out for determining them on the basis of the residual path lengths in nuclear emulsions using the threshold development mode. The stage of preliminary saturation of irradiated emulsions Type P-8 in solutions of potassium bromide with various concentrations provides the threshold development of particle tracks over a broad range of specific energy losses of gamma particles up to Ar^{40} ions.

We know that the biological effect of radiation bears a certain relationship to the energy absorbed by the tissue as

well as the linear energy losses (LEL) of the charged particle, which to a large extent determine the relative biological effectiveness of various forms of radiation. Passing through a substance, the particles of primary cosmic radiation (PCR) interact with its atoms and thereby lose a portion of their energy. The principal energy loss of the primary particles, depending on their energy, takes place either through ionization of the medium or through nuclear conversions. For a given medium, the energy loss due to ionization created by a moving particle is a function of its charge and velocity (energy).

In Figure 1 this relationship is shown for various components of the PCR in aluminum [3]. We can see from this figure that, in the case of particles with relativistic velocities, the ionization density is directly proportional to the square of the particle charge. The ionization density at the end of the path of the heavy particles increases sharply. Thus, for example,

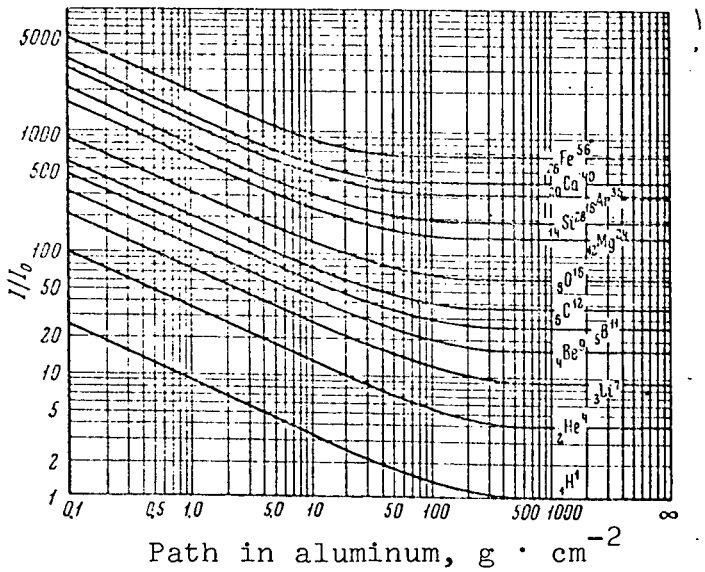

Figure 1. Specific ionization losses for various nuclei (in I_0 units — minimum ionization loss of singly charged particle) vs. their path length in aluminum

the ionization density for oxygen nuclei at relativistic velocities is 64 times greater (and at the end of the path [0.1 grams per cm^2, residual path] is 900 times greater) than the ionization density for a relativistic proton. Particularly high density of ionization is associated with the final segment of the path. In conjunction with these characteristics of the interaction of primary heavy particles with material, it has been stated [8, 11] that these particles have extremely high biological effectiveness. The effects produced by the passage of these particles through vitally important organs (for example, medulla oblongata, etc.) may be particularly serious. /364

The ionization losses of PCR particles are the most important ones at energies below 300 to 500 MeV per nucleon. At higher energies, the heavy particles mainly lose their energies in the substance as a result of nuclear reactions (formation of "stars" and showers). In the formation of a "star", a great many secondary ionizing particles are created, which in turn can produce ionization in a certain volume of material which is greater than one primary particle could produce.

During the initial period of shower formation, as a result of the nuclear collision of a primary particle with a very high energy (10^{17} eV and higher), a narrow cone of mesons may be created. Meson cones have been detected which contain several hundreds of relativistic particles. Inasmuch as the cross section of such cones in the initial stage of their development is smaller than the dimensions of a living cell, they can pass through the cell. However, the cellular dose of such beams of mesons is small in comparison to the ionization at the end of the path of heavy nuclei when stopped by ionization. The tissue dose of a beam of mesons, even in an extreme case, will be equal

to several milliroentgens in all. Therefore, from the standpoint of radiation damage, the formation of showers is of no interest to radiobiology.

Thus, for example, the 23 mesons in the narrow cone of mesons /36 produced by Rochester α-particles have a total energy of several hundred Bev, but in an individual tissue cell they would leave behind a total of approximately 1200 pairs of ions and this would take place only in the first 400 microns of the path, where the size of the cone does not exceed the size of the cell. The ordinary α-particle with an energy of several MeV is capable of forming up to 55,000 ion pairs in one cell. Heavy particles produce a much greater number of pairs of ions when they pass through a cell. This is why, in evaluating the radiation damage from primary cosmic radiation, it is necessary to determine the particle spectrum on the basis of the LEL at various depths. The latter is also necessary because the heavy particles possess significantly higher effective interaction cross sections, as a result of which the spectrum of the nuclei changes with depth.

Performing a theoretical estimate of the radiation danger of the heavy component of the PCR for a spacecraft with a real layout is practically impossible because of the difficulty of considering the changes in the particle spectrum due to their interaction with the elements of the skin and equipment of the craft, as well as the lack of data on the intensity of heavy particles with low energies. In addition, for a near-earth orbit up to altitudes on the order of 30,000 kilometers, it will be necessary to take into account the change in the spectrum as a result of the geomagnetic cutoff for various latitudes. To determine the biological effectiveness of the heavy component it is necessary to know the number of impacts of heavy nuclei on the

target and the nature of the radial distribution of the ion density in the track.

In Figure 2, which we have borrowed from Shafer [6], we have listed data on the evaluation of the doses in a track formed by Ca in tissue. It is clear from the figure that doses higher than 100 rads will be located at distances of less than 2 microns from the central portion of the track. However, according to the data of Chase [8] and Simons [10], these doses will be found at much greater distances. Thus, a dose of 100 rads according to their data will be found at a distance of 100 to 120 microns from the axis of the particle track.

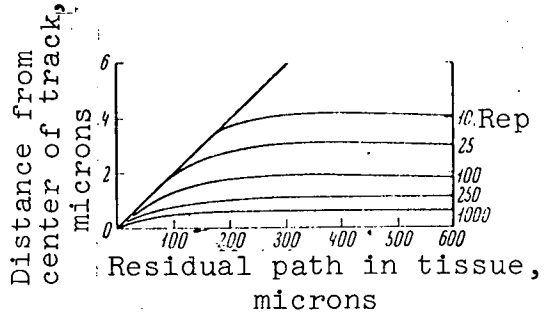

Figure 2. Radial distribution of ionization doses for tracks of heavy particles at end of path. Straight line — limit of halo of delta-electrons

As we know, there are several different methods of determining the composition of the heavy PCR component. The classical method for this purpose is based on the use of highly sensitive nuclear emulsions. Identification of the heavy fragments in such emulsions is performed with the aid of photometry (on the basis of secondary ionization), by counting the gaps and the δ-electrons, and by measuring the multiple Coulomb scattering [2].

However, this method is very laborious and involves the use of complex apparatus and qualified personnel.

Quite recently a new method of recording the heavy component of cosmic rays was developed, based on recording charged particles in dielectrics [4, 9].

Samples of dielectrics that possess various degrees of sensitivity to charged particles (mica, polycarbonate, cellulose citrate) were irradiated in outer space in the form of stacks of thin layers, and then processed (etched) in acid or alkaline solutions until hollow channels could be seen in an optical microscope.

However, this method also considerably limits the possibilities of determining particles on the basis of LEL. In addition, it makes it possible to obtain only the tracks of these particles which intersect the surface at the minimum LEL required for the etching. This is why we decided to develop a special method. It is also based on the use of nuclear emulsions, but differs as far as the processing method is concerned. Our method insures visual determination of particles with different LEL [5]. The physical basis of this method is the fact that particles with different Z charges undergo the same losses dE/dx at various distances from the end of the path. Consequently, it is necessary to select a method of treating the nuclear emulsions so that the particle tracks with different Z will have different path lengths R.

In addition, by selecting several methods of treatment with /36
various paths, we have the possibility of determining the composition of the radiation on the basis of LEL over a wide range. To carry out this task, we used fine-grain emulsions (type P-8), 200 microns thick, sensitive to protons with energies up to 5 MeV, which corresponds to the minimum losses dE/dx = 0.05 MeV/micron. The emulsions were irradiated with ions of B^{10}, N^{14}, O^{16}, Ne^{22}

and Ar^{40} with energies of 6 MeV per nucleon on the U-300 cyclotron at Dubna as well as with α-particles of ThC'.

The nuclear emulsions were processed in an iron oxalate developer [7] under conditions of controlled development. We used a developer with the following formula:

Citric acid	1.5 g
Ferrous sulfate	1.4 g
Potassium oxalate	25.0 g
10% potassium bromide	6.4 ml
Doubly distilled water	100.0 ml
pH	4.7

After the emulsion had been developed, it was washed and fixed in the usual fashion.

During the experiments with iron oxalate developer, it was found that the change in its oxidizing-reducing potential and the change in the developing process itself (developing time, temperature) did not allow us to obtain satisfactory results in terms of identifying the heavy ions, although this method did give good results [1] in recording the fission fragments with a background of α-particles, γ-rays and neutrons. Therefore, experiments were undertaken to study the role of soaking exposed nuclear emulsions in KBr solutions of different concentrations prior to development.

These experiments reveal that it is precisely the stage of preliminary soaking in potassium bromide solutions which insures the threshold appearance of particle tracks over a wide range of energy losses dE/dx from α-particles to Ar^{40} ions in P-8 nuclear emulsions. The role of KBr amounts to an increase in the

concentration of silver ions in the solution surrounding the crystals of silver bromide. Preliminary soaking in the KBr solution changes the oxidation-reducing potential of the developer, i.e., it inhibits the chemical development and accelerates the physical development of the tracks of the charged particles.

Controlled development was carried out using the methods shown in the table. It should be emphasized that the results of treatment according to these methods were reliably analyzed for each individual batch of nuclear emulsions.

It is readily apparent from the data in the table that the tracks of the heavy particles trapped in the nuclear emulsion have lengths which are determined by the charge Z and the minimal losses dE/dx at which the track is still visible. For the trapped particles, on the basis of the residual path, we can determine the charge within an accuracy of up to Z $\pm$ 2 in a region extending up to Ne. This method was tested in natural flight tests aboard the "Vostok" and "Voskhod" spacecraft. In these cases, we used nuclear emulsions (type P-8) 200 microns thick, calibrated with α-particles and heavy ions B^{10}, O^{16}, Ne^{22} and Ar^{40}. After the flight, the emulsions were developed in the fashion described above; attempts were than made to count the tracks. The emulsions calibrated with the

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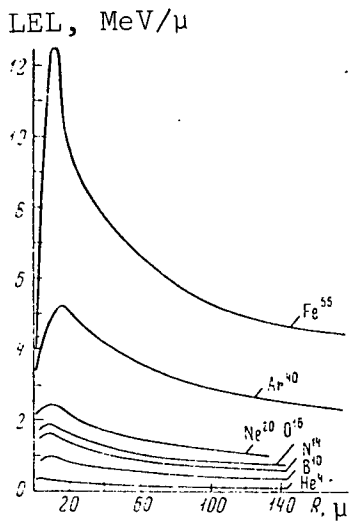

Figure 3. Linear energy losses vs. residual path of particles with various charges (Z) in emulsion

SCHEDULE OF CONTROLLED UNDERDEVELOPMENT

Soaking	Development	Discrimination threshold MeV/μ	Most easily recorded particle	Recorded residual path length of a given ion, microns
H_2O (15°, 60 min)	Amidol, 3 g Sodium sulfide, 12 g Citric acid, 2 g (15°, 60 min)	0.25	α	α 15-20
0.3% KBr (15°, 60 min)	Iron oxalate developer (15°, 60 min)	0.6	B	B 45 N 65
0.3% KBr (15°, 60 min)	Iron oxalate developer (10°, 60 min)	1.0	N	N 40 O 60
0.6% KBr (15°, 60 min)	Iron oxalate developer (10°, 60 min)	1.7	Ne	Ne 10 Ne 38
7.5% KBr (10°, 60 min)	Iron oxalate developer (10°, 90 min)	4.0	Ar	Ar 40
15% KBr (10°, 60 min)	Iron oxalate developer (10°, 90 min)	5.0	Ar	Ar 5 Fe 110

ions served as controls for the minimum losses dE/dx for each individual batch.

Thus, for example, in the case of the spacecraft "Voskhod-2" (18 - 19 March 1965), it was found with the aid of this method that the particle flux with LEL, corresponding to the LEL at the end of the path of the α-particles, amounted to 200 particles per cm^2 of a 200 micron emulsion. For boron it was 30 particles

per 1 cm^2, oxygen 15, and argon 3. The data on particles with a LEL corresponding to the LEL at the end of the paths of oxygen and argon were insufficiently accurate due to underexposure. The scanning rate in this case was equal to about 0.2 cm^2 per day. The method makes it possible to detect the heavy component of the PCR with a background of 10^{12} protons per cm^2.

Hence, this method makes it possible to carry out individual dosimetric monitoring of the most dangerous portions of the heavy component under various spaceflight conditions.

It is distinguished by simplicity of processing and measuring. We suggest that this method could also be used for identification on the basis of the charge of heavy fragments formed in the interaction of protons with nuclei under laboratory conditions.

We would like to express our deep appreciation to Academician G. N. Flerov and his associates for their kind cooperation and the opportunity that they afforded us to carry out the irradiation of the emulsions on the U-300 cyclotron at the Joint Institute of Nuclear Research in Dubna.

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Method of Combined Tissue Blocks for Comparative Pathomorphological Study of Radiation Pathology

V. G. Petrukhin and N. A. Gaydamakin

ABSTRACT. The article presents an original method developed by the authors for studying radiation pathology. On this method, an organ removed from an animal of one group is attached to filter paper together with a similar organ, removed from an animal from another group. The combined tissue block is frozen in dry ice and combined sections are then prepared in a cryostat for histochemical study. The same blocks, without preliminary freezing, are placed in corresponding fixers (solutions of formalin, Carnot,susa, etc.), dehydrated and sealed with media providing preparations for analysis by ordinary histological methods. Hence, one obtains from animals of different groups combined sections of a specific organ prepared under the same conditions.

The usual processing of material using existing histological and histochemical methods does not always permit comparative tests. Two histological preparations made from two pieces of an organ from the same animal may differ to some degree, especially in their histochemical and qualitative reactions. These differences are caused by deviations in the method of preparating a histological preparation at any stage: collecting and removing the material, preparing the sections (differences in thickness), staining and carrying out the reactions, temperature changes in

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the environment, etc. It is still more difficult to prepare completely identical preparations from two identical organs that have been taken from different animals, even if the material processing is carried out by the same laboratory technician. /370

Figure 1. Sections obtained from combined tissue blocks. 1 - adrenal (natural size); 2 - kidney; 3 - heart; 4 - small intestine; 5 and 6 - liver.

In order to eliminate differences that might possibly arise in the preparation of histological and histochemical preparations, we have devised, tested and used a method of combined tissue blocks in daily comparative studies of radiation pathology. The principal requirement of this method is simultaneous (or with intervals up to several tens of minutes) autopsy of animals from the series of experiments to be compared or animals that are being examined at different times following the same exposure.

From the autopsied animal, in a strictly determined area of the organ or the site of maximum damage, a small piece is removed and immediately placed on filter paper or on porous cardboard, which allows the fixative media, dehydration and sealing to pass through readily. A second piece is placed tightly against the first, cut from the same region of the organ of another animal, and so on.

In the experimental studies, for comparison it was important to have a standard from which the qualitative differences in the structural changes and their severity could be determined. Such a standard is usually an organ from an intact animal. Pieces of organs from intact animals were attached either to the sides of the combined block which had been formed or stacked alternately with the pieces of the organ from the experimental animals (Figure 1). A piece of some other organ must be attached to one end of the prepared block, which acts as a guide used in microscopic examination to count the sections and to determine their origin in specific animals. The arrangement of the sections in the block must be recorded in the study. If the shapes of the organs do not allow the pieces to be placed tightly one against the other — for example, when studying small laboratory animals and their adrenals, kidneys, heart or intestines — it is necessary to hold them together /371 with strips of liver around the entire parameter of the block (Figure 1). It is also desirable to have spaces between the organs filled with small pieces of liver. It is important to create a compact monolithic block of tissue. The preparation of a combined tissue block in this fashion does not require much time. The block, after it has been labeled (numbered) with an ordinary pencil (crayon) on the back, is placed in an appropriate fixer (Carnot mixture, 10% solution of formalin, susa*, etc.).

Following the first portion of 96% alcohol, the excess tissue is cut from the block and the surface is smoothed. This procedure requires care and is carried out with a well-sharpened instrument. Then the block is soaked in dehydrating fluids.

* Editor's Note: This is a decalcifying solution composed of corrosive sublimate, sodium chloride, trichloracetic acid, glacial acetic acid, formalin, and water.

Dehydration is accompanied by the pieces becoming detached along the adhesion line due to a reduction in the volume of the tissue. However, detachment of the pieces from the cardboard does not take place if the block is treated carefully. Treatment with the sealing substances makes the block monolithic once again.

On a microtome, combined sections are obtained from the surface of the block opposite the cardboard base. Further treatment and staining of the sections is carried out in accordance with ordinary methods. Preparations from combined blocks are of sufficient quality to warrant their use for cytological studies and photography at any magnification.

Almost all forms of histochemical studies are based on the comparison of the precipitation intensity of dye granules in certain cell structures. The comparison under experimental conditions is most often carried out relative to some biological control. Consequently, the histochemical studies are most advantageously carried out by preparing combined tissue blocks.

Therefore, at the time of autopsy, in addition to the block that is intended for casting in paraffin or celloidin, a second, completely identical block is prepared from each organ which is used for histochemical studies. This block is frozen in dry ice. Before sectioning in a cryostat, the block is removed from the Dewar vessel, cleaned of excess tissue in air, smoothed (not allowing it to thaw out), and frozen with the cardboard side down to the object stage of the microtome, which is located in the cryostat. The majority of tissues can be cut easily at a temperature of -6 to -12°. The sections which are obtained are fastened to a warm, degreased, chemically clean slide. The histochemical reactions on the combined sections are carried out

in accordance with the appropriate methods, differing in no way from those on a single section.

This method of combined tissue blocks for carrying out comparative histological and histochemical investigations requires careful planning of the experiments to be carried out. For convenience, we have presented one example of such /372
planning. Let us assume that it is necessary to determine the effectiveness of shielding the stomach in the case of γ-neutron radiation and to establish the differences in the damage picture in shielded and unshielded animals after 3 and 15 days. For the investigation, let us assume that in each series there will be five animals for each period, and irradiation and investigation of the animals will be carried out in subsequent periods (the animals being killed on 15 September):

Date of Irradiation	Series	No. of Animals
1 September	irradiation	1-5
12 September	ditto	6-10
1 September	shielding	11-15
12 September	ditto	16-20
	control	21-25

At the beginning, it is advantageous to kill 5 animals: 2 that were shielded and 2 that were irradiated without shielding (1 each for each period of irradiation) as well as 1 intact animal. For example, if histological and histochemical examination is to be performed on the spleen, small intestines and testes, 6 combined blocks are made up: 2 blocks each from each organ — 1 for the histological, a second for the histochemical treatment. In the first and

second blocks, there will be pieces of spleen, the third and fourth will contain intestine, and the fifth and sixth will contain testes in the order shown in diagram 14-1.

14—1	14—2	14—3	14—4	14—5
1	2	3	4	5
11	12	13	14	15
6	7	8	9	10
16	17	18	19	20
21	22	23	24	25
op.	op.	op.	op.	op.

Then the following 4 analogous groups are killed in sequence, 5 animals in each, and similar blocks are prepared (procedures 14-2, 14-3, 14-4, 14-5). Each block is given a number: the first number shows the number of the record (in the diagram, 14), the second shows the serial number of the first animal in the block. Such combined blocks from a given organ need not necessarily be 5, but more or less depending on the number of animals in each series that were killed at each observation period.

With this method, for the histological studies (or histochemical studies) it was necessary to use 15 combined blocks. However, the study of each organ individually, as is usually done, requires the preparation of 75 sections. The method makes it possible to form blocks using any other method /373
depending on the problem being investigated. If it is difficult to process all the animals in 1 day, for example, with an increase in the number of series or periods of observation, it is possible to carry out irradiation during each observation period 2 days in a row; autopsy of the animals and formation of the blocks then extends over 2 days.

If it is impossible to form a complete block immediately, assuming we are studying animals that have died at different times, the method makes it possible to have a previously frozen block stored in dry ice (this can be kept in the freezer compartment of an ordinary refrigerator) and to freeze onto it organs from animals that die on subsequent days. In order to avoid desiccation of material stored in this manner, it is necessary to keep the blocks in airtight polyethylene bags. Obviously, the principle of successive freezing of organ pieces can be used in an autopsy for comparative histochemical studies. We must not forget, however, that when an animal is autopsied it is necessary to cut out the individual pieces of the organ for storage in the event of possible additional studies. To store the material, we used penicillin flasks.

Daily experimental work, which was large in volume and the number of animals to be studied, led us to think of using sections obtained in the cryostat for successive staining by ordinary histological methods. The solution of this problem would have considerably simplified our work, and would have created still greater possibilities for carrying out comparative studies.

In the existing literature dealing with histological techniques, there are no direct mentions of this method. On the contrary, it is stated that it is impossible to use sections which were prepared from fresh-frozen unfixed tissue for histological staining. It is considered that a necessary requirement for histological treatment is preliminary fixation of the tissue. However, the sections intended for histochemical reactions must be prepared from fresh-frozen unfixed tissue, inasmuch as the passage through the fixing mixture, alcohol and sealant usually destroys or dissolves certain substances in

the tissue structures (for example, enzymes).

To determine the activity of the labile enzymes, using the existing rules for working in a cryostat, the sections are usually removed from the knife onto a warm slide. Subsequent histochemical treatment in the majority of cases is not carried out immediately, but a short time after the sectioning. Therefore, the section that is attached to the slide remains in the air for a rather long time (from several to 24 hours or more), which leads to complete desiccation of the latter. An inevitable consequence of desiccation of the /37
section is the development of artifacts due to tension, wrinkling of tissue structures, extending even to rupture of cells and changes in their shapes, as well as the formation of a protein film on the surface [5]. The film which is formed prevents loss of soluble proteins and peptides and retains the activity of the enzymes, but on the other hand it prevents penetration of reagents into the cell. Obviously this method of removing the sections from the knife and storing them, which is necessary in carrying out histochemical reactions, is completely impossible in the case of histological studies.

There are also indications in the literature that another obstacle to the use of sections obtained in the cryostat for staining by usual histological methods may be artifacts which are caused by the freezing process. A significant shortcoming of fresh unfixed tissues is the formation of ice crystals from the free water contained in the tissue spaces. Cell substances that are soluble in water may settle at the surface of the crystals. Upon freezing, there is non-uniform shrinkage of the tissue block, which may mechanically rupture the tissue and cells and form distinctive cracks.

We know, however, that the size of the crystals can be reduced by rapid freezing at low temperatures. Reducing the size of the pieces taken for tissue freezing also reduces the number of crystals formed. By regulating the size of the blocks and the cooling temperature, we can obtain sections of fresh-frozen tissue without traces of ice crystals that can be seen under the ordinary microscope [3].

The idea of using sections obtained from fresh-frozen tissue in a cryostat for the preparation of ordinary histological preparations has therefore been blocked by two principal objections: desiccation of the sections on the slide and the absence of preliminary fixation of the pieces. In order to avoid complete thawing and desiccation, it is recommended that the section be transferred from the microtome knife immediately to an appropriate incubation medium [6, 7, 9]. However, this principle was used by us to obtain sections suitable for purposes of histological staining; instead of the incubation medium, we used fixing media.

The section lying on the knife is touched lightly with a warm slide. When it straightens and thaws, it is removed together with the slide to a vessel containing a fixer that is also located in the cryostat chamber, inasmuch as it has been determined experimentally that cold fixers give the best results.

We know that in many cases good staining of histological preparations can only be achieved with a strictly governed form of fixer, so we tested many of them. It was found that fixation of cryostat sections attached to slides in cold (-5, +2°) formalin must last about an hour or more; in Carnot mixture — no more than 12 minutes; in 80 - 96% alcohol — up to 2 - 3 hours, etc.

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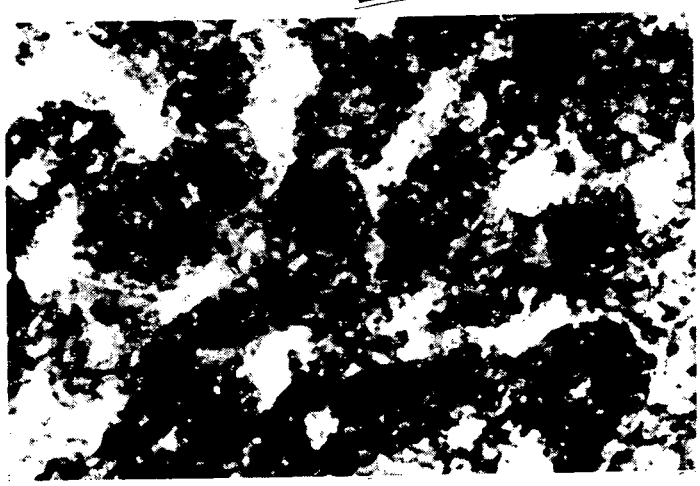

Figure 2. Liver. Photomicrograph of section prepared in cryostat from fresh-frozen tissue. Clear granules of glycogen in cell cytoplasm. Shabadash. 800 x, ob. 40.

Figure 3. Brain. Photomicrograph of section prepared in cryostat from fresh-frozen tissue. Tigroid substance in neurons. Nissl. 800 x, ob. 40.

The sections are obtained in the cryostat from fresh-frozen tissue, then directly fixed on the slide in one of the fixers, stained with eosin-hemotoxylin according to van-Gieson, scarlet red and Sudan black, using the methods of Selye and Nissl, Shabadash (to detect glycogen), Brashe (to detect RNA). We also stained with acridine orange and a number of fluorochromes used in histological studies. /377

There was practically no formation of the cracks that are associated with freezing. In preparations with reactions for glycogen, we did not see any polarization artifact ("flight from the fixer"). The glycogen granules were uniformly distributed over the entire cytoplasm. We also failed to detect any differences in the

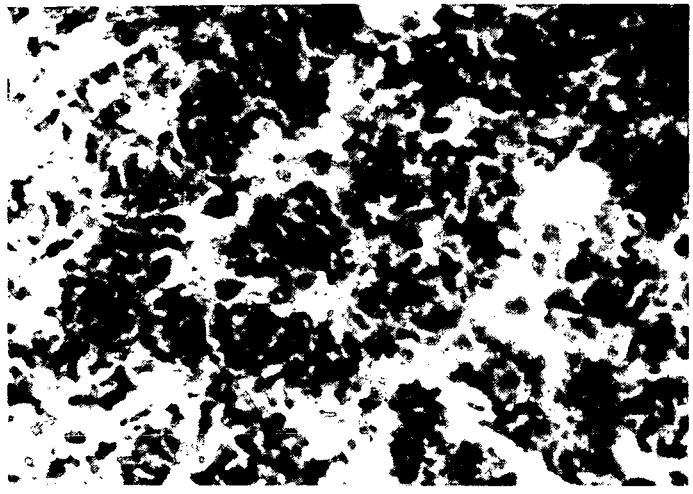

Figure 4. Liver. Photomicrograph of section prepared in cryostat from fresh-frozen tissue. Granules of RNA in cellular cytoplasm. Methyl green-pyronine. 800 x, ob. 40.

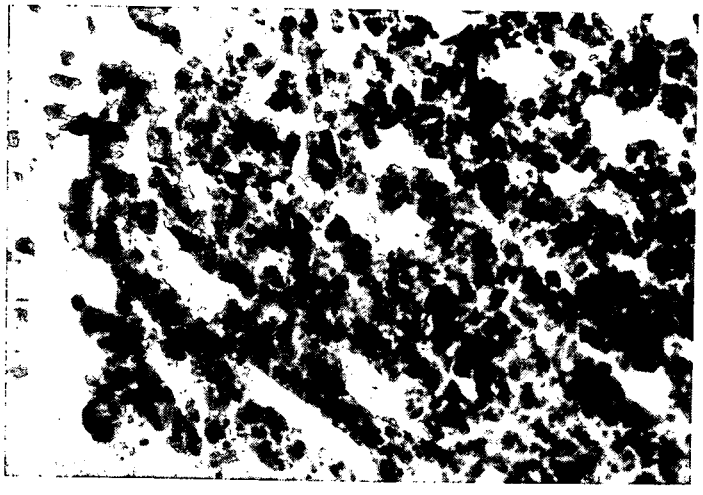

Figure 5. Adrenal. Photomicrograph of section prepared in cryostat from fresh-frozen tissue. Small and large drops of lipids in cells of nodular and reticular zones. 420 x, ob. 20.

content of glycogen in the sections that were obtained from the surface and from the depth of the block (Figure 2). The distribution of the granules of Nissl substance and RNA was uniform (Figure 3, 4). Good preparations were obtained when staining with fatty dyes (Figure 5). In this case, during the formation of the block it was not possible to leave any fatty cellulose in the capsula of the organ or tissue, since the dye could spread from the cell over the entire preparation. A shortcoming of this method is the destruction of the erythrocytes in the tissues.

We also noticed a separation of the section from the slide during fixation, especially in formalin, and

passage through staining solutions and differentiating liquids. In the majority of cases, this defect was easily overcome if we used carefully degreased dry slides. Slight desiccation of the section on the glass either during its removal from the knife or during transfer from one stain (differentiating) solution to another worsens the quality of the preparations.

Regardless of these minor shortcomings, the practical effect of the method of combined tissue blocks is completely evident. The method requires considerable time spent in collecting the material (forming the blocks). However, subsequently in the stages of fixation, dehydration, wrapping, cutting, staining and especially in carrying out the comparative studies a considerable saving of time can be achieved by the laboratory technician and his scientific associate, as compared to the use of the generally employed methods of handling the material. The presence in a single object and under one cover glass of organ sections from several animals of different series or groups, including intact animals, makes it possible to have a cross comparison between experimental animals and biological controls and also between experimental animals of different series. The absolutely identical treatment of sections and their equal thickness increases the speed and raises the reliability, convenience and accuracy of the study.

Use of sections from fresh-frozen tissue for purposes of staining by ordinary histological stains, together with the above-mentioned advantages of the method of combined tissue blocks, has other advantages as well. The sections are made from the same block as that used for the histochemical reactions. This makes it possible to compare the histological and histochemical changes in practically the same structures of the organ, which is difficult and sometimes impossible to do when

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using sections from different pieces. In addition, in many cases there is no longer a need to make up a special block for the histochemical studies, and we eliminate the long process of fixation and passing the material through the dehydrating and sealing media, which considerably shortens the volume of laboratory work. Finally, the preparations that are obtained with combined tissue blocks, in particular those treated with histochemical methods, may be subjected to mechanical analysis (for example, cytophotometry by means of scanning devices). The speed at which preparations are obtained from cryostat sections may in all probability be used in the enlarger technique, and possibly in investigating urgent biopsies.

However, this method is not universally applicable, and is not free of shortcomings, some of which are mentioned and described above.

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Problem of Relative Biological Effectiveness of Fast Neutrons

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ABSTRACT. Experiments carried out on animals showed that if the contribution of gamma radiation to the total radiation dose is not more than 10 - 15%, the level of the gamma background in approximate calculations of the RBE of the neutrons may be disregarded. With a higher contribution of gamma radiation to the total radiation dose, the error in determining the RBE coefficient increases significantly and may noticeably distort the results of the study. The paper contains a table of total doses of gamma-neutron irradiation for two values of the gamma background, which facilitates the determination of the neutron RBE coefficient with allowance for the contribution of gamma radiation to the dose.

The determination of the relative biological effectiveness /378
(RBE) of various forms of ionizing radiation, including fast neutrons, is one of the most important problems in radiobiology. As a "standard" to which we can compare the biological effect of neutrons and other forms of radiation, we usually use γ-radiation from radioactive cobalt Co^{60} (the average energy of the gamma rays is 1.25 MeV) or x-radiation (150 - 250 keV). The biological /379
effectiveness of these types of radiation is assumed to be unity.

The majority of authors determine the relative biological effectiveness as the ratio of the amounts of absorbed energy of

γ-(x) radiation and the investigated form of radiation with equal biological action. We know that the biological effectiveness of nuclear radiation depends on the spatial distribution of the energy in the irradiated organism, liberated along the track of an ionizing particle (photon), i.e., on the linear ionization density. But the latter depends, in turn, on the form (nature) of the radiation. This is why a nonuniform biological effect may correspond to equal quantities of absorbed energy of different types of radiation.

At the present time, a number of papers have been written dealing with the effectiveness of fast neutrons, but thus far there has been no uniform opinion concerning the RBE coefficient for this type of radiation. Various authors give values for the RBE of fast neutrons which sometimes differ by several factors of 10. These discrepancies are explained by the fact that the RBE of any type of radiation is not a constant value characterizing all reactions of the organism to radiation. The nature and magnitude of the biological effect produced by the action of radiation is influenced by a number of factors: the species, sex and strain of the animal selected for the experiment, the test (index) selected for evaluating this effect, the time which has elapsed from the time of exposure until the time of reading of the results obtained, the accuracy of determining the radiation dose and the energy of irradiation, the nature of the radiation (acute or chronic), the presence and magnitude of γ-background and a number of other factors.

Naturally such diverse factors acting on the RBE have not always been taken into account by all of the investigators, and are the reason for certain contradictions that sometimes are very important in the results that were obtained by individual scientists at different times. Let us dwell briefly on the

significance of certain of the factors mentioned above.

Curtis in 1951 [21] showed that the relative biological effectiveness of fast neutrons, calculated from the dose of half survival (LD_{50}), is practically the same for mice, rats and rabbits of both sexes ($\sim$3). However, it was established in the studies of many authors that the value of the RBE coefficient of fast neutrons determined by a single test may have different values not only for animals that belong to different species, but also for various strains of the same species.

According to the data of Cogan et al. [20] the RBE of fast neutrons with respect to the formation of cataracts amounts to 9 - 11 for rabbits, and 4 - 5 for mice. Niri, Manson and Mol [4] found that the RBE of fast neutrons calculated from the decrease in weight of the testes has different values for 3 genetically different strains of mice: for the SVA strain — 5.1, for F_1 (A X SVA) strain — 6.2, and for the S 57 bl strain — 9.7. They did find, however, that the RBE for neutrons (as far as the decrease in fertility of mice is concerned) is equal to 6 - 7 for the males and 3 for females of the same strain. /380

Sverdlov, Mozzhukhin and others [5] have shown that, in the case of neutrons with an energy of 2.5 MeV, the RBE coefficient determined on the basis of the dose which produces death of all irradiated animals ($LD_{100/30}$) is equal to 2.8 for mice, 3.1 for rats and 2.0 for guinea pigs.

A matter of very great significance for the evaluation of the RBE coefficient is the test which is selected in each specific instance. The RBE coefficient of fast neutrons, calculated on the basis of the dose of half survival or on the basis of the dose that leads to the death of all the animals, according to

the data of many authors, is equal to 2 - 3 on the average [5, 9, 21, 27, 35, 36], although individual studies carried out basically at earlier times give still greater values for this index. Evans [25, 26] found the value of the RBE coefficient for fast neutrons to be 8, while Clark et al. [18, 19] and Vogel et al. [39] found it to be 4.4. A few authors [17, 22, 24, 32] feel that the RBE of fast neutrons, on the basis of the acute mortality of the animals, is less than 2 (1.6 - 1.8).

The authors mentioned above measured the RBE of fast neutrons on the basis of the acute mortality of the animals with a 30-day observation period. However, there are convincing data which indicate that the RBE coefficient varies greatly as a function of the observation periods that have elapsed since the time of irradiation with the fast neutrons. Even Curtis in 1951 [21] showed that all the mice that survived exposure to fast neutrons at a dose equal to $LD_{50/30}$ will die during the subsequent nine months, which is not observed to the same degree for γ-radiation. This is why the RBE coefficient of the neutrons, calculated on the basis of the mortality of the animals that do not die during the first 30 days, will have a higher value at later times.

The studies of Vogel et al. [40] also mention the significance of the time factor, the period which has elapsed since the moment of irradiation until the moment of recording the experimental results. He and his associates studied the mortality of 4-day chick embryos following irradiation with fast neutrons and γ-rays. The RBE coefficient of the neutrons, when the living embryos were counted 6 hours following irradiation, was equal to 2.2. When they were counted after 6 days, it was higher and approached 3.

It should also be mentioned that, with the same final outcome — for example, survival to the 30^{th} day following irradiation — the radiation damage in the acute period following the action of fast neutrons has a more serious nature, the recovery processes proceed more slowly, and aftereffects are observed more frequently than with γ-radiation [5 - 8, 10, 19, 27, 39].

In the case of irradiation with neutrons in particular, the /381
acute intestinal syndrome is much more evident, and therefore a relatively greater part of the irradiated animals will die at early stages from "acute intestinal death". According to the data of Clark et al., [19] Vogel et al., [39] with identical mortality on the 30^{th} day, the majority of the mice, following irradiation with neutrons in the fission spectrum, will die on the 5^{th} or 6^{th} days, while after γ-irradiation they will die on the 13^{th} through 14^{th} days. In our experiments on rats that were subjected to radiation in doses that exceeded the minimum absolute lethal dose by 50 - 100 rads, the majority of animals died after irradiation with fission neutrons (more than 85%) in the period from the 3^{rd} through the 6^{th} days, while after γ-irradiation (about 70%) — in the period from the 7^{th} through the 11^{th} days. This is why the average survival time (AST) of the animals which died following irradiation with neutrons was shorter than under the influence of γ-rays with the same survival rate by the 30^{th} day.

In the experiments of Hiss [27], performed on rats that were subjected to the action of neutrons and γ-rays in absolute minimum lethal doses, the AST of the animals was 6.5 and 12.5 days, respectively. In our experiments also performed on rats, the AST of the animals following exposure to fission neutrons at a dose of $LD_{75/30}$ was 8.4 days, while after γ-irradiation at the same dose it was 11.5 days. In the case of irradiation

at the minimum absolute lethal dose ($LD_{100/30}$), this same index was equal to 4.9 and 8.4 days, respectively.

The characteristics of the clinical course of radiation damage produced by fast neutrons provide a good explanation for the increase in the RBE determined on the basis of the mortality index of the animals during the first 5 - 6 days following the exposure, i.e., during the period of acute intestinal death, in comparison with the coefficient calculated on the basis of the mortality to the 30^{th} day. According to the data of Sverdlov et al., [5], the RBE coefficient for mice determined on the basis of the $LD_{100/6}$ is equal to 4, while on the basis of $LD_{100/30}$ it is 2.8. In the studies on mice irradiated with neutrons having energies of 1.9 - 0.8 MeV, Davids [23] obtained an RBE 1.9 with consideration of mortality from the "marrow syndrome", while with consideration of mortality from the "intestinal syndrome" it was 3.0.

Some investigators have used, as the criterion for evaluating the RBE of fast neutrons, the index of the average survival time of the irradiated animals that survive after the 30^{th} day. According to the data of Curtis [22], the RBE coefficient of neutrons according to this index does not differ from that for acute mortality of mice and is equal to 1.7. At the same time, however, Storer et al., [35] have shown that the RBE of neutrons on the basis of the AST of mice is somewhat higher than according to the index $LD_{100/30}$, and is equal to 2.6 instead of 2.3. Apton et al., [38], using the same tests in experiments on mice, give still higher values for the RBE coefficient — from 3 to 10 depending on the radiation energy.

Sometime ago, data were accumulated [11, 25, 26, 28, 30, 31, 41] indicating that certain animal organs and tissues

have particularly pronounced sensitivity to neutron radiation. /382
These organs include in particular the eye (eyeball, crystal lens), the reproductive and hemopoietic organs, and also the intestine. This is why, in the evaluation of the biological effectiveness of neutrons by the degree of damage to these organs and tissues, the RBE coefficient is higher as a rule than when the indices of acute mortality of these animals were used.

It has been known for a long time that the eye is highly sensitive to the effects of neutrons. According to the data which we already mentioned (Cogan et al., [20]), as far as the ability to develop cataracts is concerned, the RBE of fast neutrons is 4 - 5 to 9 - 11 for various animals. Apton et al. [37] gives slightly different numerical indices for the RBE coefficient on the basis of this same test. According to their data, the effectiveness of fast neutrons with respect to the formation of cataracts in mice, rats and guinea pigs is only 3 to 4 times higher than for γ-rays.

At the same time, however, Bateman et al., [14] comparatively recently showed once again the high cataractogenic capacity of fast neutrons. In the case of neutrons with energies of 0.43 and 1.8 MeV, with respect to early cataractogenesis in mice, the RBE is roughly 10 times higher than for x-rays (250 keV).

Bateman, Johnson et al. [15] used the determination of the effective doses of neutrons and γ-rays at which the number of resting spermatogonia in the seminal vesicles of the testes of mice decreased by 50% in the evaluation of the RBE. They found that, in the case of neutrons with different energies, the RBE coefficient varied from 2.5 to 10.3 on the basis of this index.

Yamamoto and Osamu [42] determined the RBE coefficient of neutrons (14.1 MeV) in comparison to x-rays (180 keV) on the basis of the weight loss in the reproductive organs of mice, and found that in the case of males — in terms of the weight drop of the testes by 50% — the RBE equals 2.7 - 2.8, while in females on the basis of the weight loss of the ovaries it was 3.2.

Spaulding et al. [34] used the index of fertility of male mice subjected to the action of neutrons and energies of 1.4 and 14 MeV to evaluate the RBE. They found that on the basis of this index the RBE of neutrons with energies of 1.4 MeV is approximately 6.5 times higher than for γ-rays, while for neutrons with an energy of 14 MeV there is practically no difference with respect to that for γ-radiation.

In studying the RBE of neutrons, many investigators have used as a test the decrease in weight (atrophy) of the spleen, thymus, and sometimes the small intestine. According to the data of Carter, Bond and Seymour [17], the RBE of neutrons on the basis of the weight drop of the spleen and thymus does not differ essentially from this index calculated from the mortality of the animals (mice) by the 30^{th} day following irradiation and is equal to (on the basis of spleen atrophy) 1.7; atrophy of the thymus — 1.5; and on the basis of acute mortality — 1.6. It is only on the basis of the index of the /383
weight decrease of the small intestine that the RBE of the neutrons, according to their data, is somewhat higher and is equal to 2.3. Approximately the same value (1.8) for the RBE coefficient of the fission neutrons on the basis of the spleen weight loss was obtained by Storer et al. [35].

At the same time, however, in a number of studies higher values of the RBE for neutrons were obtained, calculated on the

basis of the weight loss of these same organs. According to the data of Jordan et al. [29], Clark et al. [19] and Vogel et al. [39], fission neutrons on the basis of the index of the spleen weight decrease by 50% are 4.4 times more effective than γ-rays. Bateman et al. [13] obtained values for the RBE coefficient for neutrons with energies of 1.8 MeV (on the basis of the spleen atrophy index) of 3.3 and on the basis of the thymus atrophy index — of 3.0. For neutrons with energies of 40.33 MeV, it was 4.6 and 4.2, respectively.

An important role in the determination of neutron RBE is played by factors which relate to the dosimetric characteristic of the radiation. We have in mind not only the correct measurement of the dose rate in the radiation dose, but also the determination of the distribution of the radiation energy absorbed in the irradiated tissues, the evaluation of the energy of the neutrons themselves, the contribution of the γ-component to the total radiation dose, etc.

An incorrect determination of the neutron radiation dose will lead to gross errors in the evaluation of the relative biological effectiveness of this type of radiation. For a rather long time, dosimetry of neutrons has also made use of the so-called n-unit. However, an inadequate definition of this unit has led to an incorrect determination of the energy absorbed by the biological tissue and an increase in the RBE values of fast neutrons [2].

The same viewpoint was held by Curtis and Gebhard [22]. They feel that in evaluating the material on the basis of the RBE of the neutrons obtained by the authors, who used n-units for measuring the doses of this type of radiation, it is necessary to double the doses expressed in n-units. In their

paper, Curtis and Gebhard include a table in which the RBE values for the fast neutrons obtained in early studies by various authors on the basis of the acute mortality of the animals are compared with their own data, using the coefficient 2 which they propose. In particular, in examining the experimental material published by Evans [25, 26], they feel that, if the correction which they propose is taken into account, the RBE coefficients of the fast neutrons calculated by the author on the basis of the $LD_{50/30}$ for mice will be equal to 4, and not 8.

In studying the relative biological effectiveness of neutrons, sources of neutron radiation were used which emit neutron fluxes of different energy. But, as has been shown in a number of different studies, the energy of the neutrons has a considerable influence on the RBE coefficient. According to the data of the overwhelming majority of investigators, the RBE of /384
fast neutrons decreases with an increase in their energy. Only in the work of Carter et al. [17] was a change in the RBE of the neutrons not noticed (on the basis of the survival and atrophy of the spleen, thymus and small intestine) with an increase in their energy from 3 to 20 MeV.

Riley, Evans et al. [33], in experiments on mice, established that their RBE falls with an increase in the neutron energy: on the basis of the index of acute mortality, on the 30^{th} day it decreases from 2.7 to 1.2, and on the basis of cataractogenesis — from 5.0 to 2.5. In the papers by Spaulding and others cited above [34] and those of Bateman et al. [13], a decrease in the RBE of neutrons with an increase in their energy was also pointed out.

Berendsen et al. [12], in experiments on a culture of human kidney cells, determined the RBE of fast neutrons on the basis of the 50% survival of the irradiated cells and found a drop in the RBE from 4.5 to 2.3 with an increase in the neutron energy from 2.7 to 15 MeV. In the paper by Bateman et al. mentioned above [15], the drop in the RBE coefficient of fast neutrons from 10.3 to 2.5 with an increase in the radiation energy from 0.43 to 14 MeV was also shown. As we can see from these data, with considerable variations in the value of the neutron energy, the relative biological effectiveness of this radiation can change by a factor of 2 to 4 and more.

The RBE value of the neutrons is also greatly influenced by the duration of the irradiation, which is unavoidably linked to the change in the radiation dose rate and the number of times the influence is imposed (multiple irradiation). It has been shown that in the case of rapid lethal reactions in acute experiments, the RBE will be less than for chronic exposure at small doses. On the basis of the data of Evans [25], Curtis [21], Niri, Manson and Mol [4], Shal'nova [9] et al., the RBE of fast neutrons with chronic irradiation with doses of small intensity will vary from 3.6 to 30 depending on the object used, the test adopted by the writers, and the radiation dose rate.

In the experiments of Clark, Jordan and Vogel [18] it was shown that, as the duration of the irradiation increases by a factor of 16 (from 1.5 to 24 hours), the RBE of the fast neutrons (based on the $LD_{50/30}$ index for mice) will increase from 4.4 to 5.8. Anton et al. [38] irradiated mice with neutrons with energies of 1 and 5 MeV, as well as gamma (x) rays, at the same time changing the dose rate over a wide range. The RBE of the neutrons, calculated on the basis

of the survival time of the animals, increased parallel to the drop in the dose rate, was equal to approximately 3 at the maximum dose rate (85 rads per minute), and exceeded 10 at the lowest dose rate (3 10^{-6} rads/minute), as a result of the drop in the biological effectiveness of gamma rays at low dose intensities.

Bailey et al. [16] subjected the skin of pigs to single and multiple irradiation with fast neutrons and x-rays, and found an increase in the RBE coefficient of the neutrons from 2.3 for a single irradiation to 3.6 with 15 irradiations.

The biological effect of irradiation with fast neutrons /385
depends not only on the magnitude of the absorbed dose and its rate, but also on the spatial distribution of the absorbed energy in the irradiated organism. The significance of this factor was clearly demonstrated in the original experiment of Gorodetskiy, Kirichinskiy and Chebotarev [1]. Three groups of rats were subjected to irradiation with fast neutrons at the same dose. However, the rats in the first group were irradiated in rather large containers, in which they could assume any position. The rats in the second and third groups were held in place during irradiation, so that the lengthwise axis of the body in the rats of the second group coincided with the direction of the neutron beam. In the rats of the third group, it was perpendicular to the direction of this beam.

The mortality of the animals in the first 30 days was 56.5 in the first group, ten in the second and 83% in the third. Radiation sickness was mild in the rats of the second group, but it was particularly serious in the rats of the third group. The results obtained are explained by the authors as being due to the rapid decrease in the intensity of the

neutrons even after passing through a comparatively small thickness of biological tissue, due to the latter's high hydrogen content.

By means of dosimetric studies, the authors of this paper established that in the rats of the second group the dose in the region of the abdominal cavity amounts to only 50% of the dose in the region of the head, and at the end it amounted to only about 30%. At the same time, however, when rats in the third group were irradiated they observed nearly uniform distribution of the doses over parts of the body. The dose along the midline of the body amounted to 80% of the dose at the mouth.

Consequently, in the case of irradiation with fast neutrons, in order to obtain uniform results it is necessary to have uniform orientation of the animals with respect to the irradiated beam, which is achieved by strict fixation of the animals or by designing specific conditions for placing them in all of the groups of the experiment which are to be compared.

We know that the overwhelming majority of neutron sources give out gamma rays along with the neutrons. At a small value of the gamma component (gamma background) in an approximate evaluation of the RBE it is possible in some cases to disregard its contribution to the radiation dose. However, frequently the contribution of gamma radiation to the absorbed dose turns out to be highly significant, and has a considerable influence on the correct determination of the relative biological effectiveness of the neutrons.

Levin [3] feels that in radiobiological investigations it is necessary to use neutron fluxes in which the gamma background

does not exceed 20%. Unfortunately, in many radiobiological papers devoted to the study of the biological effectiveness of neutrons, there are no data on the gamma background.

The present paper presents some data on the relative biological effectiveness of fission neutrons obtained in experiments on rats that were subjected to gamma-neutron irradiation with different values of the gamma component.

The experiments were performed on sexually mature white /386
random-bred rats of both sexes, kept under ordinary vivarium conditions on a standard diet. The principal criteria for evaluating the biological effects were the survival indices up to the 30^{th} day following irradiation, and the average survival time of the rats which died under the influence of lethal and superlethal doses of radiation. The animals were subjected to irradiation with a gamma neutron flux on a research uranium reactor under two modes of exposure: in the first mode the contribution of neutrons to the absorbed dose (neutron component) was approximately 90%, while in the second it was only 35%. Consequently, in the first case the gamma component amounted to only 10% in all and was of no importance. In the second, the contribution of the gamma component to the total radiation dose amounted to 65%.

The dose rate of the gamma-neutron flux in the first mode amounted to 12 rad/min, while in the second it was about 30. The principal contribution to the neutron dose (about 80%) came from the fast neutrons ($E > 0.4$ MeV), and the thermal neutrons made up only about 1% in all. The average energy of the neutrons was equal to about 1 MeV, while that of the gamma rays was 1.6 - 1.8 MeV. For gamma irradiation, a device was used with a Co^{60} charge. The dose rate of gamma

radiation at a distance of 1 m from the source was 12 - 15 r/min.

In the first experiments, the animals were subjected to gamma-neutron irradiation with a small contribution of gamma rays to the absorbed dose. Therefore, for the simplicity of the calculations, we can still disregard the contribution of the gamma component to the radiation dose, and can conditionally assume that the animals were subjected to the action of the neutron flux alone. We used doses that produced mortality of 75 and 100% of the irradiated animals ($LD_{75/30}$ and $LD_{100/30}$). Some of the rats were subjected to gamma radiation over a dose range of 850 - 1200 r. The results of the experiment are shown in Table 1.

As we can see from the table, an equal biological effect in terms of survival by the 30^{th} day was obtained with gamma-neutron irradiation of the rats over a dose range of 350 - 380 rads and gamma radiation in a dose of 850 r. This means that the dose of gamma-neutron radiation (350 - 380 rads) was equivalent to the dose of gamma radiation (850 r) in terms of biological effectiveness.

As we know, the unit of tissue dose which takes into account the corresponding correction for the biological effectiveness of a specific species of ionizing radiation is the biological equivalent of the rad (Rem). The tissue dose in Rems is determined by multiplying the dose (in rads) by the RBE coefficient of this type of radiation:

$$D_{(Rem)} \approx D_{(rad)} \cdot RBE \qquad (1)$$

We will conditionally assume that for gamma rays the dose in rads is approximately equal to the dose in roentgens, and /387

TABLE 1

SURVIVAL AND AVERAGE SURVIVAL TIME OF ANIMALS WHICH DIED FROM VARIOUS DOSES OF GAMMA RADIATION AND GAMMA-NEUTRON FLUX WITH A GAMMA COMPONENT OF ABOUT 10%

Radiation	Dose	No. animals		Survival, % ± m	AST of rats that died, days, M ± m	RBE_n	
		Total	Survived			w/o consideration of gamma background	w/ consideration of gamma background
Gamma rays, Co^{60}	850 r	32	8	25.0±7.7	11.5±1.0	—	—
	900 r	20	0	0	9.4± 0.9	—	—
	1000 r	60	0	0	8.4±0.3	—	—
	1200 r	24	0	0	4.8±0.4	—	—
Gamma-neutron flux	350 — 380, rads	30	7	23.3±7.7	8.4±0.7	2.2 — 2.4 (2.3)	2.4 — 2.6 (2.5)
	450 — 500, rads	70	0	0	4.9±0.2	2.2 + 2.4 (2.3)	2.3 — 2.6 (2.45)

therefore the RBE of this type of radiation is taken as unity, so that the tissue dose of the gamma rays expressed in Rems is also approximately equal to the dose in roentgens. It follows from Equation (1) that, for the specific case with which we are dealing, the dose of neutrons (in Rems) will be equal to:

$$D_{n(Rem)} \approx 350(380) \cdot RBE_n \qquad (2)$$

and for gamma rays the dose (in Rems) will be

$$D_{\gamma(Rem)} \approx 850. \qquad (3)$$

The relationships given above indicate that an equal biological effect — with neutron radiation in doses of 350 - 380 rads and gamma radiation at a dose of 850 r — indicates equal tissue doses expressed in Rems:

$$D_{n(Rem)} \approx D_{\gamma(Rem)} \qquad (4)$$

From Equations (2), (3) and (4) it follows that:

$$350(380) \text{ rads} \times RBE_n = 850 \text{ Rems}$$

Consequently, the RBE coefficient of the neutrons will be /388 equal to:

$$RBE_n = \frac{850}{350(380)} = 2.2 — 2.4$$

In the same fashion, we can determine the RBE coefficient of the neutrons on the basis of the dose that leads to death of all irradiated animals. On the basis of the data from our experiments, the minimum lethal dose of gamma radiation for

rats is about 900 r, and that of neutrons is 400 - 420 rads. In experiments with irradiation of rats with gamma-neutron flux, a dose of 450 - 500 rads was used, which is about 50 - 100 rads greater than the minimum lethal dose (see Table 1).

As was shown by observations of the clinical course of radiation sickness and its aftereffects, the biological effect of a gamma-neutron flux in doses of 450 - 500 rads was slightly higher than the effect of gamma radiation at a dose of 1,000 r. Therefore, with a certain degree of accuracy, we can assume that the biological effect of neutron radiation at these doses is equivalent to the effect of gamma radiation at a dose of approximately 1,100 r. The RBE coefficient of the neutrons will be equal to approximately 2.2 - 2.4 in this case, just as when $LD_{75/30}$ is used. Hence, the experimental estimate of the relative biological effectiveness of the neutrons, essentially speaking, leads to the establishment of the biologically equivalent doses of gamma radiation and neutron flux on the basis of the index selected in each specific case (test), after which the determination of the RBE coefficient will not pose any particular difficulty.

However, the values we obtained for the RBE coefficient of the neutrons — calculated from the survival index of the animals on the 30^{th} day — is somewhat reduced, since in the calculations that were performed, we did not take at least two factors into account.

First: as we have already said earlier, with the same final outcome of the experiment — in this case, survival up to the 30^{th} day with irradiation of neutrons — the severity of the damage was great, while the average survival time of the animals which were killed was less than with gamma radiation in

biologically equivalent doses. In our experiments, with doses of gamma-neutron radiation of 350 - 380 and 450 - 500 rads, the AST of the rats which died was approximately the same as with gamma radiation at doses of 1,000 and 1,200 r, respectively, i.e., at much higher doses than was the case when evaluating the biological effect on the basis of severity on the 30^{th} day (850 and 1,100 r). Therefore, the RBE of the neutrons, taking into account the seriousness of the damage and the AST index of the animals which died, will be somewhat higher than when using the survival index alone.

Second: in analyzing the results of the experiments, for the sake of simplicity we did not take into account the contribution of the gamma component to the total dose of gamma neutron radiation, inasmuch as it was only about 10 - 12%. As simple calculations using this contribution show, the significance of the RBE coefficient of the neutrons increases insignifi- /389
cantly in this case, by 10 - 15% in all.

However, a significant increase in the gamma background, as indicated by the experimental data, can lead to serious errors in the evaluation of the RBE of the neutrons, if we do not take into account the contribution of the gamma component to the total dose of radiation. The influence of the gamma background on the RBE coefficient of the neutrons was reliably demonstrated in an experiment with irradiation of rats at two values of the gamma-component. In the first case, its contribution to the total dose of gamma-neutron radiation, just as in the previous experiments, amounted to only 10 - 12% in all, while in the second it was much higher, about 65%.

It is completely understandable that in order to attain equal biological effectiveness the total radiation dose of the

animals at the second value of the gamma component, when there is a sharp drop in the contribution of neutrons to the absorbed dose, must be higher than in the first. However, it was difficult to determine the value of this dose in advance. This is why we used three doses to irradiate the animals: 500, 590 and 680 rads, exceeding the radiation dose with a considerable contribution of neutrons (350 rads) by 1.4, 1.7 and 2 times (Table 2).

It must be pointed out that in this experiment, involving irradiation of rats with a dose of 350 rads, the biological effect was slightly higher than in the preceding experiment (see Table 1). We can explain this result as being due primarily to errors in the neutron dosimetry methods used in this experiment. However, we know that the methods used at the present time can have a measurement error of up to $\pm$20%. With a high biological effectiveness of the neutrons, such an error can lead to the fact that in many cases, with exactly the same value of the measured dose being 350 rads, the actual value of the radiation dose can be 50 - 70 rads higher or lower, which leads in one case to survival of a certain portion of the irradiated animals, and in the other — to their complete death.

However, for a comparative evaluation of the results of this experiment, the relative measurement error of the neutron flux was not of critical importance, inasmuch as the entire experiment was performed simultaneously, with uniform irradiation conditions and measurements of the doses of gamma-neutron flux received by the animals.

The results of the experiment showed that a uniform biological effect was obtained when rats were irradiated with a high contribution of neutrons in a dose of 350 rads, and with

TABLE 2

SURVIVAL AND AVERAGE SURVIVAL TIME OF ANIMALS WHICH DIED AFTER GAMMA-NEUTRON IRRADIATION WITH TWO VALUES OF THE NEUTRON COMPONENT

Contribution of neutrons to dose, %	Radiation dose, rads	No. of rats		AST of rats which died, days M ± m
		Total	Survived	
90	350	30	0	4.5±0.2
35	500	10	0	7.0±0.6
	590	20	0	5.1±0.6
	680	10	0	4.1±0.1

a small contribution of neutrons at a dose of about 600 rads. Approximately the same biological effect was observed in the case of gamma irradiation of animals at a dose of approximately 1,100 r.

If, in the first experiments, we do not take into account the contribution to the dose of gamma-radiation, and we assume conditionally that the total dose of radiation expressed in rads is formed only by the neutron flux, the relative biological effectiveness of the neutrons relative to the gamma radiation from the Co^{60} in the first case (with a low gamma-background) will be equal to about 3.1 (1,100:350), and in the second (with a high gamma-background) it will be approximately 2 (1,100:600). /390 The first number is close to the true value of the RBE coefficient of the neutrons used in this experiment inasmuch as the low gamma background did not have a significant influence on its value. However, the second value of the coefficient is definitely reduced, inasmuch as it does not take into account the sharp change in the ratio of the contribution of neutrons and gamma rays to the dose, to the advantage of the gamma rays.

The experiment we performed made it possible to determine the RBE coefficient of fast neutrons, with allowance for the correction for the gamma-background, through comparison of two biologically equivalent doses of gamma-neutron irradiation, expressed in Rems, received by animals in two versions of the neutron contribution to the total dose. For this purpose, a table was drawn up which shows the changes in the total doses of gamma-neutron radiation expressed in Rems as a function of the ratio between the contribution of neutrons and gamma rays to the total absorbed dose (in rads) and the values assumed for the RBE of the neutrons (Table 3).

TABLE 3

TOTAL DOSES OF GAMMA-NEUTRON RADIATION (IN REMS) AS A FUNCTION OF THE RATIO BETWEEN THE CONTRIBUTIONS OF NEUTRONS AND GAMMA RAYS TO THE ABSORBED DOSE AND THE ASSUMED VALUES OF THE NEUTRON RBE COEFFICIENT

Index	RBE	Ratio of contribution to absorbed dose of neutrons and gamma rays, %						
		n/γ = 90/10				n/γ = 35/65		
Total absorbed dose of gamma-neutron flux, rads	—	350	400	450	500	500	590	680
Dose of gamma rays, rads	—	∿40	40	40	∿50	50	320	440
Dose of neutrons, rads	—	310	360	400	450	180	210	240
Dose of neutrons with different values of RBE coefficient, Rems.	1.5	460	540	600	675	270	315	360
	2.0	620	720	800	900	360	420	480
	2.5	770	900	1000	1125	450	525	600
	3.0	930	1080	1200	1350	540	630	720
	3.5	1080	1260	1400	1575	630	735	840
	4.0	1240	1440	1600	1800	720	840	960
Total dose of gamma-neutron flux at various values of RBE coefficient, Rems.	1.5	500	580	650	725	590	700	800
	2.0	660	760	850	950	689	800	920
	2.5	810	940	1050	1175	770	900	1040
	3.0	970	1120	1250	1400	860	1000	1160
	3.5	1120	1300	1450	1625	950	1100	1280
	4.0	1280	1480	1650	1850	1040	1220	1400

Using this table, it is easy to see that, with an equivalent biological effect, in the case of gamma-neutron irradiation of the animals at a dose of 350 rads with a high value of the neutron component and at a dose of 600 rads with a small neutron contribution, the total radiation doses expressed in Rems can only be equal when the RBE coefficient of the neutrons has a value between 3.0 and 3.5.

By means of the same table, we can also determine the RBE coefficient of neutrons with consideration of the gamma background, through comparison of the biological equivalent doses of gamma radiation of Co^{60} and gamma-neutron flux. We have already said that the biological effect for gamma-neutron radiation at a dose of 350 rads with a high neutron component, and at a dose of 600 rads with a low neutron component, is approximately the same as with gamma irradiation from Co^{60} at a dose of approximately 1,100 r. This means that all three doses, expressed in Rems, must be approximately the same. It is evident from Table 3 that the radiation dose will have a value equal to 1,100 Rems when the RBE coefficient of the neutrons is equal to approximately 3.5 both with a high and a low background of gamma radiation. This value of the neutron RBE coefficient is slightly different, approximately 13 - 15%, from that calculated through direct comparison of the equivalent doses of gamma radiation and gamma-neutron flux, without consideration of a low gamma background, and almost two times higher than the coefficient calculated in the same fashion at a high gamma-background (3.5 instead of 2). /392

Hence, the experiments which we performed clearly demonstrated that, in studying the RBE of fast neutrons, it is necessary to take into account the gamma-radiation accompanying the neutron flux. If the contribution of gamma radiation to the total radiation dose is small, no more than 10 - 15%, the gamma

background in approximate calculations of the neutron RBE may be disregarded, inasmuch as the error will not exceed 20 - 25%. At higher values for the contribution of the gamma radiation to the total radiation dose, the error in determining the RBE coefficient of the neutrons increases significantly and may distort the results significantly.

In our experiments, to irradiate the rats we used a gamma-neutron flux that was emitted by a uranium reactor following the use of lead filters and without preliminary filtration. In the first case, the contribution of the gamma radiation to the total radiation dose amounted to about 10 - 12%, and in the second — to about 65%.

The table which we have compiled showing the total doses of gamma-neutron radiation for the two gamma background values will make it possible to determine easily the neutron RBE coefficient, allowing for the contribution to the dose of the gamma radiation accompanying the neutron flux. This coefficient may be determined in two ways: through comparison of two biologically equivalent doses of gamma-neutron radiation, received by the animals with different contributions of gamma rays to the absorbed dose, or through the direct comparison of one of the total doses of gamma-neutron radiation with a biologically equivalent dose of gamma radiation from Co^{60}. However, similar tables including other possible values of the gamma-background may be recommended in studies of the biological effectiveness of neutrons.

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Excretion of Free Amino Acids with the Urine as a Test for Early Diagnosis of Radiation Damage

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ABSTRACT. In the case of 30 individuals who were subjected to combined radiation therapy for treatment of cancer of the uterus, the author studied the excretion of free amino acids with the urine and the change in their level in the blood as a function of the total dose of radiation during treatment by the method of univariate partition paper chromatography. The increase in amount of several free amino acids in the urine is an early and sensitive indicator of radiation action on the human organism.

There are many reports in the literature that mention that /394 excretion of amino acids with the urine is a sensitive indicator of radiation effects on the human organism. Initially, a significant increase in the quantity of all amino acids in the urine in eight individuals with acute radiation sickness of serious and moderate degree was observed by using partition paper chromatography [1]. The authors felt that such intensive amino aciduria is not observed in any other ailment. A very thorough study of excretion of amino acids with the urine in four individuals who were injured in a nuclear reactor accident was performed by Katz and Hasterlik [9]. The victims received comparatively small doses of γ radiation and a neutron flux of about 25 to 180 r. In three individuals, there was a complete lack of any kind of clinical-hematological data that would

indicate the presence of radiation damage, and only one had mild symptoms of radiation sickness. An analysis of the urine in all of these victims and a chromatographic determination of the amino acids commenced during the first hours following irradiation, lasted for 16 days of hospitalization, and then was continued for four months more in three of the individuals. An increase in the excretion of amino acids was found even 12 hours after exposure. The maximum amino aciduria in all four patients was observed on the sixth day following irradiation and lasted four months. The total daily excretion of amino acids increased on the average by a factor of 10: excretion of individual amino acids varied from a two- to twenty-fold increase in comparison with the norm. The sharpest rise was in the excretion of glycine and hydroxyproline. The relationships between the dose of radiation and the rate of amino acid excretion could not be established. Small doses of radiation, in the opinion of the authors, preclude the possibility of a significant breakdown of proteins. However, intensive excretion of amino acids involves a decrease in oxidation and utilization of the latter in the liver and other tissues as well as the disruption of readsorption in the renal tubules. The authors feel that the change in the quantity of amino acids in the urine may be a sensitive and early indicator of metabolism disturbance at small radiation doses.

In subsequent investigations, data obtained by these authors were supported partly or completely, although the results of some of the reports were contradictory in nature. Thus, Kretchmer [11], who studied eight individuals who were irradiated with moderate doses of γ-radiation (clinical symptoms were insignificant) as the result of a reactor accident at Oak Ridge, /395 observed a decrease in the excretion of serine with the urine of the victims during the first day, and almost complete

disappearance of this substance on the third day following exposure. The increase of the quantity of β-aminoisobutylic acid (an amino acid that is involved in DNA metabolism) in the urine of the irradiated individuals was noticed by Rubini et al., [14], Gerber et al., [3] and Andrews et al., [2]. It was found that the excretion rate of this acid increased in proportion to the radiation dose. Iammet [8] and Pendic [13] found a significant increase in excretion of all amino acids with the urine in six individuals who were irradiated with a dose of 350 - 640 rads as the result of an accident with an experimental nuclear reactor in Yugoslavia. Howland et al. [5] as well as Gamis, Hendrickson and Howland [4] described the disturbance of amino acid excretion in six individuals who were subjected to the action of high doses of radiation during an accident with a nuclear installation at Lockport. All of the patients showed amino aciduria. The most significant factor in four patients out of six was an increase in excretion of cystine, prolite and tryptophan. The authors noticed a relationship between the increase in amino acid excretion and the development of erythema in the patients. Excretion of free amino acids with the urine was also studied in the case of irradiation of experimental animals [6, 7, 10, 12].

Hence, an analysis of the material in the literature shows that the majority of authors find the change in excretion of free amino acids with the urine to be a response to irradiation. However, the experimental data from various investigators are contradictory and corresponding clinical observations are very scarce. In view of the fact that changes frequently arose during radiation therapy in these patients that were very similar to radiation sickness of a mild degree, we set ourselves the task of studying the excretion of amino acids with the urine, as well as the changes in the level of these substances in the

blood during radiation treatment as a function of the radiation dose.

We studied 30 individuals in all, ranging in age from 30 to 65, who were subjected to combined radiation therapy for treatment of cancer of the uterus. External irradiation was administered with four fields, two iliac and two sacral, measuring 10 x 15 cm on the x-ray-therapeutic apparatus (RUM-3) under the following technical conditions: 180 - 1 - 8 kV; 10 - 15 mA; filter — 0.5 mm Cu + 1 mm Al, layer for half-attenuation — 1 mm Cu, skin-focus distance — 50 cm. The individual dose per field was 223 r, with two fields being irradiated each day. Treatment began with external irradiation, combined after 10 - 12 days with intracavitary radiation. The preparations of cobalt with an activity of 6.4 to 33.6 mg equivalents of radium were administered in an intra-uterine manner for 46 hours, 3 to 5 times with intervals of 6 to 7 days. With this combined method, the doses from external x-radiation varied from 14,378 to 21,760 r (absorbed at the depth of the parametrium on each side from 1,820 to 2,700 rads), from the intracavitary — at point A from 6,730 to 11,670 rads; at point B — from 1,700 to 5,000 rads. /396

In the course of radiation treatment, 13 individuals developed symptoms of radiation sickness of mild degree, twelve showed scattered symptoms of radiation reaction and only 5 individuals completed the course of x-ray radiotherapy without signs of radiation damage.

An analysis of the free amino acids was performed with the aid of a univariate partition paper chromatography. We investigated the daily excretion of amino acids and their level in the blood of all the patients before the start and after the end of radiation treatment. In addition, in the case of ten

individuals we made analyses on the second, fifth, tenth, twentieth — twenty-fifth and forty-fifth — fiftieth days of irradiation. This enabled us to establish the dependence of the disturbances in the amino acid metabolism on the total dose of radiation. We carried out a qualitative determination of eighteen free amino acids in 200 samples of blood and urine.

It was established that at the end of the x-ray radiotherapy, the patients showed an increase in the level of free amino acids in the blood and an increase in their excretion with the urine (see the table). These changes turned out to be much more distinct in patients with symptoms of radiation sickness than in those persons who finished the treatment without signs of radiation sickness. /397

It is evident from Figures 1 and 2 that the concentration of free amino acids in the blood during the first days of irradiation did not change. Only on the 20 - 25th days, when the total dose from external irradiation reached 9,000 - 10,000 r (on all four fields) was there a moderate increase in the level of several amino acids.

However, the greatest changes in the blood were observed at the end of the radiation therapy.

The change in the excretion of amino acids in the urine differs significantly from the changes in its level in the blood. An increased excretion of amino acids showed up earlier and was caused by smaller doses of radiation. Thus, on the 5th day of radiation treatment (total dose of local irradiation amounted to only 1,800 r) the concentration of serine increased on the average (in comparison to the original level) by 105%; that of

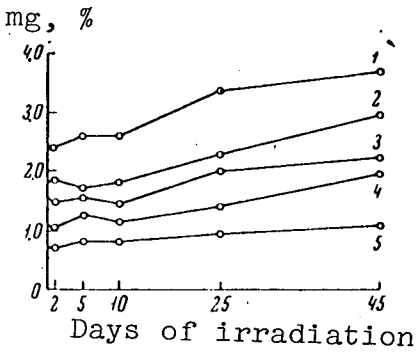

Figure 1. Concentration of certain amino acids in the blood of patients during radiation therapy. 1 - cystine; 2 - glutamine; 3 - methionine + valine; 4 - aspartic acid; 5 - histidine

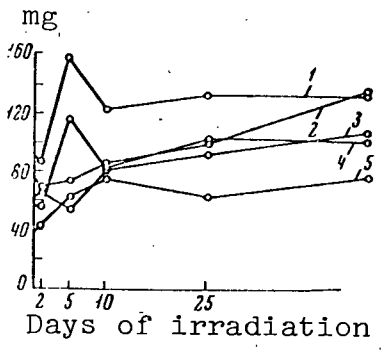

Figure 2. Dynamics of the excretion of certain amino acids with urine during radiation therapy. 1 - cystine; 2 - glycine; 3 - methionine + valine; 4 - serine; 5 - aspartic acid.

glutaminic acid — by 93%;
glycine — by 79%; and
aspartic acid — by 55%.

How can we explain the difference in the levels of amino acids that developed in the blood and urine of the patients during the period of radiation therapy? We know that excretion of individual amino acids with the urine depends mainly on two factors: their concentrations in the blood and readsorption in the renal tubules. Hence, we know that even slight changes in the blood chemistry will have an effect /398 on the chemical composition of the urine, since by increasing or reducing the excretion of certain substances with the urine, the organism can retain a constant blood composition. It is this obviously which causes the initial increase in the number of free amino acids in the urine. Then at higher doses of radiation, when the compensatory capacities of the kidneys are exhausted, an increase in the level of amino acids in the blood takes place.

TABLE

CONTENT OF FREE AMINO ACIDS IN THE BLOOD (IN mg %) AND IN DAILY URINE (IN mg) IN 30 PATIENTS (M ± m) WITH CANCER OF THE UTERUS PRIOR TO START AND AT END OF RADIATION THERAPY.

Amino acids	Blood			Urine		
	Before treatment	At end Of treatment	Difference, %	Before treatment	At end Of treatment	Difference, %
Lysine	1.22±0.09	1.03±0.07	-14	27±4	34±5	+25
Treonine	1.40±0.10	2.00±0.14	+43*	61±4	73±6	+19**
Tryptophan	0.90±0.06	0.84±0.05	- 6	45±4	52±6	+15**
Valine + methionine	1.50±0.14	2.30±0.21	+53*	55±6	91±8	+66**
Phenylalanine	1.60±0.13	1.78±0.09	+12	23±3	24±4	+ 4
Leucine + isoleucine	1.23±0.08	1.38±0.16	+12	15±2	17±3	+13
Glutaminic acid	0.89±0.05	1.31±0.16	+47*	30±3	56±5	+86**
Glutamine	2.04±0.18	3.18±0.25	+56*	35±4	60±6	+71**
Alanine	1.69±0.13	2.43±0.19	+44*	40±4	77±6	+92**
Aspartic acid	1.09±0.11	1.82±0.20	+76*	48±6	76±8	+57**
Cystine	2.32±0.20	3.52±0.24	+52*	62±9	122±15	+96**
Histidine	0.78±0.06	1.12±0.09	+43*	30±4	49±7	+63**
Arginine	1.44±0.10	1.43±0.14	- 1	25±3	32±6	+28
Glycine	1.97±0.14	2.89±0.21	+43*	84±7	141±11	+67**
Serine	1.18±0.15	1.74±0.16	+47*	52±55	87±7	+67**
Thyrozine	1.0 ±0.06	1.13±0.08	+13	39±5	46±7	+18

* $p < 0.01$.

** $p > 0.05$.

It must be emphasized that during the first five to ten days of irradiation no clinical or hematological signs of radiation damage can be found in the patients as a rule. However, excretion of certain amino acids with the urine at this time has already increased significantly.

Hence, an analysis of this material indicates that the change in the amount of free amino acids in the urine is an early and sensitive indication of radiation influence on the human organism. Therefore, a study of urinary excretion of amino acids may be used for diagnosis of radiation damage.

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